Electronic Supplementary Material (ESI) for Chemical Communications

Exploiting Higher Alkynophilicity of Au-Species: Development of Highly Selective Fluorescent Probe for Gold Ions

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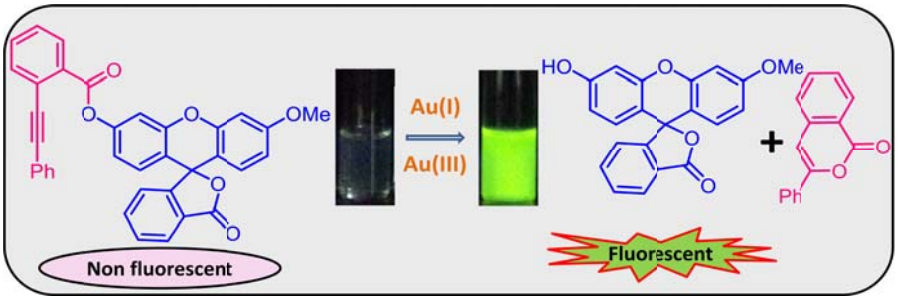


Contents

1. Introduction

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified and dried by standard methods prior to use. Twice-distilled water was used throughout all experiments. The solutions of metal ions were prepared from $HAuCl_4.3H_2O$, AuCl, AgOTf, $Ba(OH)_2.8H_2O$, $FeCl_3.8H_2O$, $Bi(OTf)_3$ $Hg(OTf)_2$, $IrCl_3$, KI, $La(OTf)_3$, $MnSO_4 \cdot H_2O$, $NiCl_2 \cdot 6H_2O$, $CdCl_2$, $PdCl_2$, $PtCl_2$, $PtCl_4$, $RuCl_3$, $CrCl_3$, $Sc(OTf)_3$, $Yb(OTf)_3$, $ZnCl_2$.

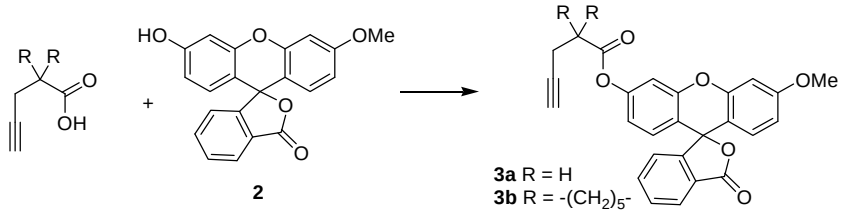
All reactions were carried out in oven or flame dried vials with magnetic stirring under nitrogen atmosphere. Dried solvents and liquid reagents were transferred by oven-dried syringes or hypodermic syringe cooled to ambient temperature in a desiccators. All experiments were monitored by analytical thin layer chromatography (TLC). TLC was performed on pre-coated silica gel plates. After elution, plate was visualized under UV illumination at 254 nm for UV active materials. Further visualization was achieved by staining $KMnO_4$ and charring on a hot plate. Solvents were removed in vacuo and heated with a water bath at 35 °C. Silica gel finer than 200 mesh was used for flash column chromatography. Columns were packed as slurry of silica gel in hexane and equilibrated with the appropriate solvent mixture prior to use. The compounds were loaded neat or as a concentrated solution using the appropriate solvent system. The elution was assisted by applying pressure with an air pump.

Melting points are uncorrected. IR spectra were recorded as KBr pellets and absorptions are reported in cm^{-1}. ^{1}HNMR spectra were recorded on 300 and 500 MHz spectrometers in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. Multiplicities of ^{1}HNMR signals are designated as s (singlet), brs (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplet), m (multiplet)… etc. ^{13}C NMR spectra were recorded on 75 MHz

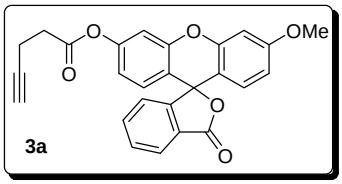
spectrometers. High-resolution mass spectra were obtained by using ESI-QTOF mass spectrometry.

2. Organic Synthesis:

2.1 General procedure for preparation compound 3a and 3b:



To a mixture of alkynoic acid (1.0 equiv.), EDC (1.0 equiv.) and DMAP (0.1 equiv.) in DCM was added fluorescein **2** (1.0 equiv.) in DCM at rt. The mixture was stirred for additional 12 hrs. After completion of reaction the solvent was removed under reduced pressure and resulting residue was purified by flash column chromatography with 10/90 mixture of ethyl acetate and hexane as eluent to yield pure products.



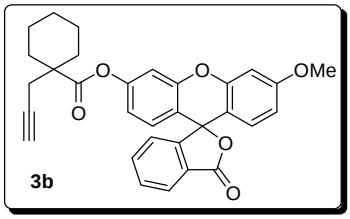
(**3a**): 79% yield; white solid; mp = 138-141°C; R_f 0.50 (hexane/EtOAc = 60/40).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04-8.02 (d, *J* = 7.5 Hz, 1H), 7.71-7.60 (m, 2H) 7.18-7.16 (d, *J* = 7.5 Hz, 1H), 7.1 (s, 1H), 6.81 (m, 2H), 6.78-6.77 (d, *J* = 2.3 Hz, 1H), 6.72-6.69 (d, J=8.3 Hz, 1H), 6.65-6.61 (dd, *J* = 8.3 & 2.3 Hz, 1H), 3.84 (s, 3H), 2.85-2.80 (t, *J* = 7.5 Hz, 2H), 2.66-2.60 (t, *J* = 7.5 Hz, 2H), 2.05-2.04 (t, *J* = 2.3 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 169.2, 161.4, 152.9, 152.2, 151.8, 151.7, 135.1, 129.8, 129.0, 128.9, 126.4, 125.0, 117.3, 116.8, 111.9, 110.8, 110.2, 100.8, 82.3, 81.8, 69.5, 55.5, 33.4, 14.4.

IR (film): ν_{max} 2927, 1762, 1598, 1477, 1419, 1245, 1102, 835 cm^{-1}.

MS (ESI) *m/z* 427.0 (M^+ + H); HRMS calcd for $C_{26}H_{18}O_6$ (M^+ + H) 427.11816 found 427.11819.



(**3b**): 83% yield; white solid; mp = 179-182°C; R_f 0.45 (hexane/EtOAc = 60/40).

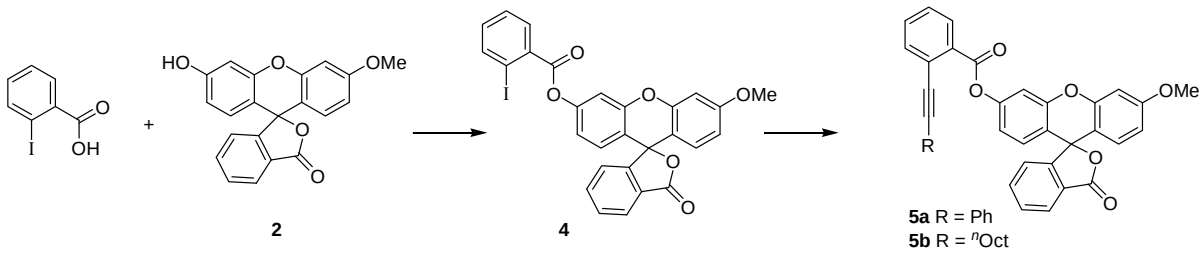
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04-8.02 (d, *J* = 6.8 Hz, 1H) 7.70-7.60 (m, 2H), 7.18-7.15 (d, *J* = 7.5 Hz, 1H), 7.07 (s, 1H), 6.80-6.77 (m, 3H), 6.72-6.70 (d, *J* = 8.3 Hz, 1H), 6.65-6.61 (dd, *J* = 9.1 & 2.3 Hz, 1H), 3.84 (s, 3H), 2.57-2.56 (d, *J* = 2.3 Hz, 2H), 2.26-2.22 (m, 2H), 2.1 (s, 1H), 1.65-1.49 (m, 6H), 1.39-1.24 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): 173.6, 169.3, 161.4, 153.0, 152.2, 151.8, 135.0, 129.8, 128.9, 126.4, 125.0, 123.9, 117.5, 116.6, 111.8, 110.8, 110.4, 100.8, 82.4, 79.7, 71.4, 55.5, 47.0, 33.2, 30.9, 29.6, 29.2, 25.4, 22.8.

IR (film): ν_{max} 2931, 1764, 1601, 1497, 1423, 1247, 1167, 1107, 884, 759 cm^{-1}.

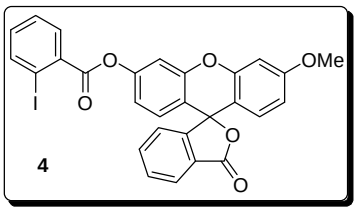
MS (ESI) *m/z* 495.0 (M^+ + H); HRMS calcd for $C_{31}H_{26}O_6$ (M^+ + H) 495.18076 found 495.18072.

2.2 General procedure for preparation compound 5a and 5b:



Procedure for synthesis of ester 4:

Ester **4** was prepared by following similar procedure mentioned for the preparation of **3a** and **3b** as described in section 2.1.



(**4**) 68% yield; pale yellow solid; mp = 155-158°C; R_f 0.60 (hexane/EtOAc = 60/40).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08-8.00 (m, 3H), 7.69-7.58 (m, 3H) 7.50-7.47 (t, *J* = 7.0 Hz, 1H), 7.23-7.14 (m, 2H), 6.96-6.86 (dd, *J* = 8.9 & 2.0 Hz, 1H), 6.79-6.76 (dd, *J* = 8.9 & 2.0 Hz, 2H), 6.69-6.72 (d, *J* = 8.9 Hz, 1H), 6.61-6.59 (dd, *J* = 8.9, 2.0 Hz, 1H), 3.83 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2, 164.3, 161.2, 152.2, 151.8, 141.7, 135.1, 134.9, 133.5, 131.6, 129.8, 129.6, 129.1, 129.0, 128.1, 125.0, 124.9, 123.9, 123.8, 117.4, 11.9, 111.5, 110.4, 100.7, 94.7, 55.5.

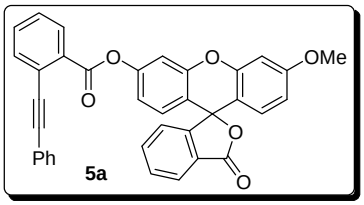
IR (film): ν_{max} 2924, 1762, 1609, 1500, 1423, 1245, 1166, 1106, 878, 759 cm^{-1}.

MS (ESI) *m/z* 577.0 (M^+ + H); HRMS calcd for $C_{28}H_{17}IO_6$ (M^+ + H) 577.01481 found 577.01487.

General procedure for synthesis of probes 5a and 5b:

To a solution of ester **4** (1.0 equiv.) in anhydrous THF (5 mL) were added CuI (0.025 equiv) and PPh_3 (0.025 equiv) in a flame dried two neck flask with magnetic stirring bar and rubber septum. The mixture was degassed and filed with nitrogen three times and then $PdCl_2(PPh_3)_2$ (0.05 equiv), Et_3N (1.5 equiv), and phenylacetylene or octyne (1.5 equiv) were added to the solution. The reaction mixture was stirred for 12 h at rt and then filtered through a pad of silica and Celite. The solvents were removed under reduced pressure and the crude oil obtained was purified by chromatography on silica gel, using a 10/90 mixture of ethyl

acetate/hexane as the eluent to yield corresponding product **5a** and **5b** in 78 and 82% yields, respectively.



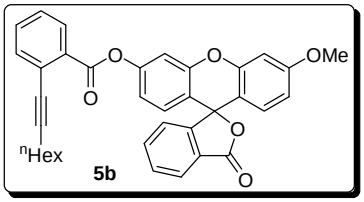
(**5a**) 78% yield; brownish solid; mp = 185-187°C; R_f 0.60 (hexane/EtOAc = 60/40);

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.18-8.16 (d, *J* = 7.7 Hz, 1H), 8.05-8.03 (d, J = 7.7 Hz 1H), 7.74-7.72 (d, *J* = 7.7 Hz, 1H), 7.71-7.68 (t, *J* = 7.7 Hz, 1H), 7.65-7.62 (t, *J* = 7.7 Hz 1H), 7.61-7.58 (t, *J* = 7.7 Hz, 1H), 7.52-7.50 (m, 2H), 7.49-7.46 (t, *J* = 7.7 Hz, 1H), 7.52-7.50 (m, 2H), 7.49-7.46 (t, *J* = 7.7 Hz, 1H), 7.33-7.31 (m, 3H), 7.28-7.27 (d, *J* = 2.2 Hz, 1H), 7.22-7.20 (d, *J* = 7.7 Hz, 1H), 6.98-6.96 (dd, *J* = 8.8 & 2.2 Hz, 1H), 6.87-6.85 (d, *J* = 8.8 Hz, 1H), 6.80-6.79 (d, *J* = 2.2 Hz, 1H), 6.73-6.71 (d, *J* = 8.8 Hz, 1H), 6.66-6.63 (dd, *J* = 8.8 & 2.2 Hz, 1H), 3.85 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): 169.3, 161.4, 152.9, 152.2, 152.1, 135.1, 134.3, 132.6, 131.7, 131.0, 129.8, 129.1, 128.9, 128.6, 128.3, 128.0, 126.4, 125.0, 124.5, 124.0, 122.9, 117.5, 116.7, 111.9, 110.4, 100.8, 95.3, 82.5, 55.5.

IR (film): ν_{max} 2924, 1764, 1609, 1497, 1421, 1105, 1031, 756 cm^{-1}.

MS (ESI) *m/z* 551.0 (M^+ + H); HRMS calcd for $C_{36}H_{22}O_6$ (M^+ + H) 551.14946 found 551.14946.



(**5b**) 82% yield; white solid; mp = 169-171°C; R_f 0.65 (hexane/EtOAc = 60/40).

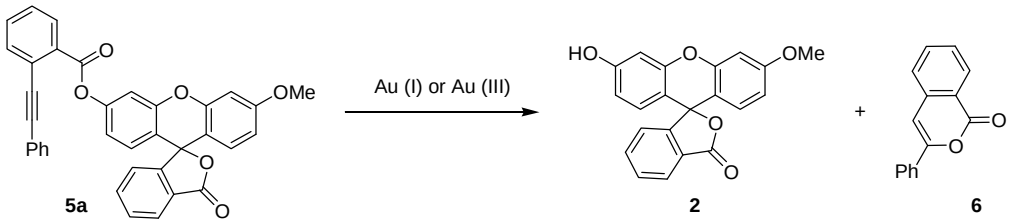
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10-8.03 (m, 3H), 7.72-7.61 (m, 3H), 7.53-7.48 (t, *J* = 7.5 Hz, 1H), 7.25-7.18 (m, 2H), 6.98-6.94 (dd, *J* = 8.3 & 2.3 Hz, 1H), 6.89-6.86 (d, *J* = 8.3 Hz, 1H), 6.80-6.79 (d, *J* =1.5 Hz, 1H), 6.74-6.71 (d, *J* = 8.9 Hz, 1H), 6.66-6.62 (dd, *J* = 8.9 & 2.3 Hz, 1H), 3.85 (s, 3H), 3.77-3.73 (t, *J* = 6.0 Hz, 2H), 1.87-1.83 (m, 2H), 1.35-1.20 (m, 6H), 0.92-0.83 (m, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): 161.4, 153.0, 152.2, 151.9, 151,8, 141.7, 135.1, 133.5, 131.6, 130.8, 129.9, 129.2, 129.0, 128.8, 128.1, 126.4, 125.1, 124.0, 117.4, 117.0, 112.0, 110.8, 110.4, 110.2, 100.8, 94.7, 68.1, 55.6, 30.3, 30.0, 23.7, 22.7, 14.0, 10.9.

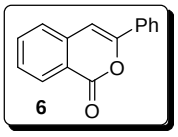
IR (film): v_{max} 3386.9, 2924, 1704, 1486, 1452, 1345, 1205, 1157, 942, 751 cm^{-1}.

MS (ESI) *m/z* 559.0 (M^+ + H); HRMS calcd for $C_{36}H_{30}O_6$ (M^+ + H) 559.21206 found 559.21216.

2.3 Test Reactions



To a screw-cap vial containing stir bar, were added probe **5a** (1.0 equiv.), and catalyst $HAuCl_4.3H_2O$ or AuCl (0.01 equiv.) in aq. CH_3CN (2 mL/mmol). The reaction vial was fitted with a cap and stirred at rt for 1 hr. The solvent was removed under reduced pressure and resulting residue was purified by flash column chromatography using hexane and ethyl acetate as eluent to obtain flurophore **2** and isochromene-1one **6**.



3-Phenyl-1H-isochromen-1-one: 94% yield; white solid; mp = 89-91°C; R_f 0.65 (hexane/EtOAc = 60/40).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33-8.30 (d, *J* = 7.5 Hz, 1H) 7.91-7.90 (d, *J* = 7.5 Hz, 1H), 7.88-7.87 (d, *J* = 7.5 Hz, 1H), 7.75-7.69 (dd, *J* = 7.5 & 1.5Hz, 1H), 7.53-7.43 (m, 5H), 6.96 (s, 1H).

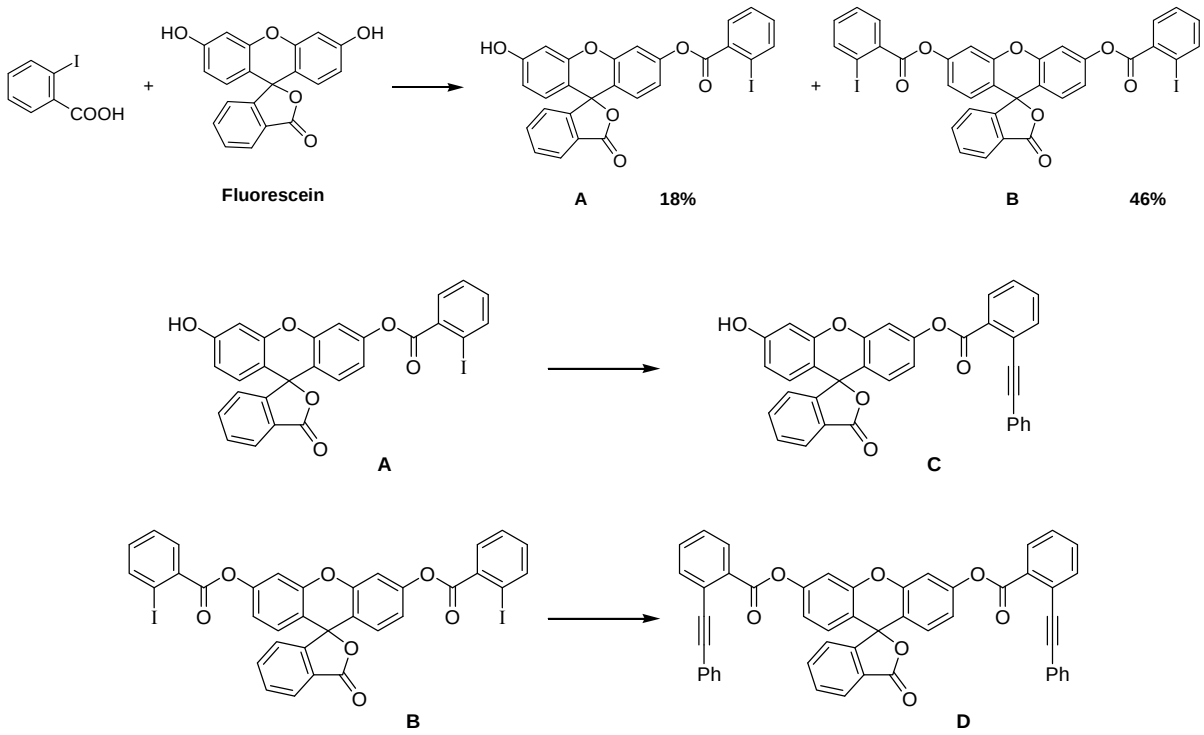
^{13}C NMR (75 MHz, $CDCl_3$): 162.3, 153.5, 137.4, 134.8, 131.9, 129.9, 129.5, 128.8, 128.1, 125.9, 125.2, 101.8.

IR (film): ν_{max} 3069, 3027, 2962, 1732, 1631, 1483, 1234, 1069, 766 cm^{-1}.

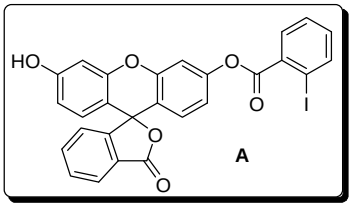
MS (ESI) *m/z* 223.0 (M^+ + H); HRMS calcd for $C_{15}H_{10}O_2$ (M^+ + H) 223.07542 found 223.07561.

2.4 Preparation compound C and D:

One of the referees suggested that the methoxy-fluorescein **2**, is not good fluorophore for imaging purpose because of its much weaker fluorescence compared to fluorescein itself. Accordingly, we prepared probe **C** and **D** as depicted below.



Esters **A** and **B** were obtained from the reaction between fluorescein (1.0 equiv.), 2-iodobenzoic acid (3.0 equiv.), EDC (3.0 equiv.) and DMAP (0.5 equiv.) by following similar procedure mentioned for the preparation of **3a** and **3b** as described in section 2.1.



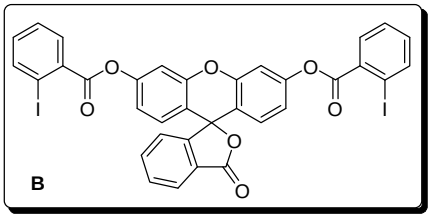
(**A**) 18% yield; yellowish white solid; mp = 198-200°C; R_f 0.40 (hexane/EtOAc = 60/40).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09-8.06 (t, *J* = 8.0 Hz, 2H), 8.05-8.02 (t, *J* = 8.0 Hz, 2H), 7.72-7.69 (t, *J* = 8.0 Hz, 1H), 7.67-7.64 (t, *J* = 8.0 Hz, 1H), 7.51-7.48 (t, *J* = 8.0 Hz, 2H), 7.30-7.29 (dd, *J* = 8.0 & 2.0 Hz, 1H), 7.24-7.21(t, *J* = 8.0 & 2.0 Hz, 2H), 7.02-7.00 (dd, *J* = 9.0 & 2.0 Hz, 2H), 6.93-6.91 (d, *J* = 9.0 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): 169.2, 164.3, 153.0, 152.0, 151.6, 141.8, 135.3, 133.5, 131.6, 130.1, 129.1, 128.1, 125.9, 125.3, 124.0, 117.8, 116.8, 110.5, 94.8, 81.5.

IR (film): ν_{max} 3387, 1743, 1599, 1587, 1481, 1427, 1235, 1019, 889, 734 cm^{-1}.

MS (ESI) *m/z* 563.0 (M^+ + H); HRMS calcd for $C_{27}H_{15}O_6$ (M^+ + H) 563.30887 found 563.30899.



(**B**) 46% yield; yellowish white solid; mp = 205-207°C; R_f 0.70 (hexane/EtOAc = 60/40).

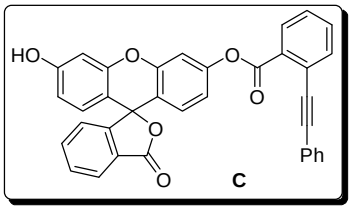
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10-8.04 (m, 5H), 7.75-7.64 (m, 2H), 7.52-7.48 (t, *J* = 6.8 Hz, 2H), 7.30-7.21 (m, 5H), 7.02-6.99 (m, 2H), 6.94-6.91 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): 169.1, 164.2, 153.0, 152.3, 152.0, 151.5, 141.7, 135.3, 133.5, 131.6, 130.1, 129.0, 128.1, 125.9, 125.2, 124.0, 117.8, 116.7, 110.5, 94.7, 81.5.

IR (film): ν_{max} 1747, 1608, 1580, 1489, 1421, 1231, 1011, 889, 737 cm^{-1}.

MS (ESI) *m/z* 793.0 (M^+ + H); HRMS calcd for $C_{34}H_{18}I_2O_7$ (M^+ + H) 793.31940 found 793.31937.

Compound **C** and **D** were prepared by following similar procedure mentioned for the preparation of compound **5a** and **5b** as described in section 2.2, except for **D** where mole equivalents for all reagents were doubled.



C

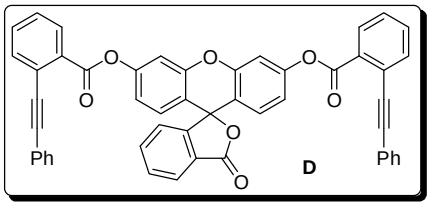
(**C**) 83% yield; white fluppy solid; mp = 105-107°C; R_f 0.35 (hexane/EtOAc = 60/40).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19-8.16 (dd, *J* = 7.9 & 1.3 Hz, 2H), 7.74-7.71 (m, 2H), 7.62-7.57 (dt, *J* = 7.5 & 1.3 Hz, 1H), 7.50-7.49 (d, *J* = 3.4 Hz, 2H), 7.33-7.27 (m, 8H) 7.03-6.99 (dd, *J* = 8.7 & 2.3 Hz, 2H), 6.91-6.88 (d, *J* = 8.5 Hz, 2H) .

^{13}C NMR (75 MHz, $CDCl_3$): 169.1, 164.0, 152.3, 151.7, 134.3, 132.6, 131.7, 131.1, 130.3, 130.0, 129.0, 128.7, 128.3, 128.0, 126.1, 125.2, 124.6, 124.1, 122.9, 117.9, 116.5, 110.6, 95.4, 95.1, 86.8.

IR (film): ν_{max} 3450, 1768, 1748, 1610, 1493, 1233, 1154, 756 cm^{-1}.

MS (ESI) *m/z* 537.0 (M^+ + H); HRMS calcd for $C_{35}H_{20}O_6$ (M^+ + H) 537.53764 found 537.53771.



D

(**D**) 74% yield; white solid; mp = 99-101°C; R_f 0.60 (hexane/EtOAc = 60/40).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10-8.04 (m, 6H), 7.75-7.66 (m, 3H), 7.53-7.48 (m, 4H), 7.34-7.27 (m, 6H), 7.25-7.21(m, 3H), 7.03-6.99 (m, 3H), 6.94-6.89 (m, 3H).

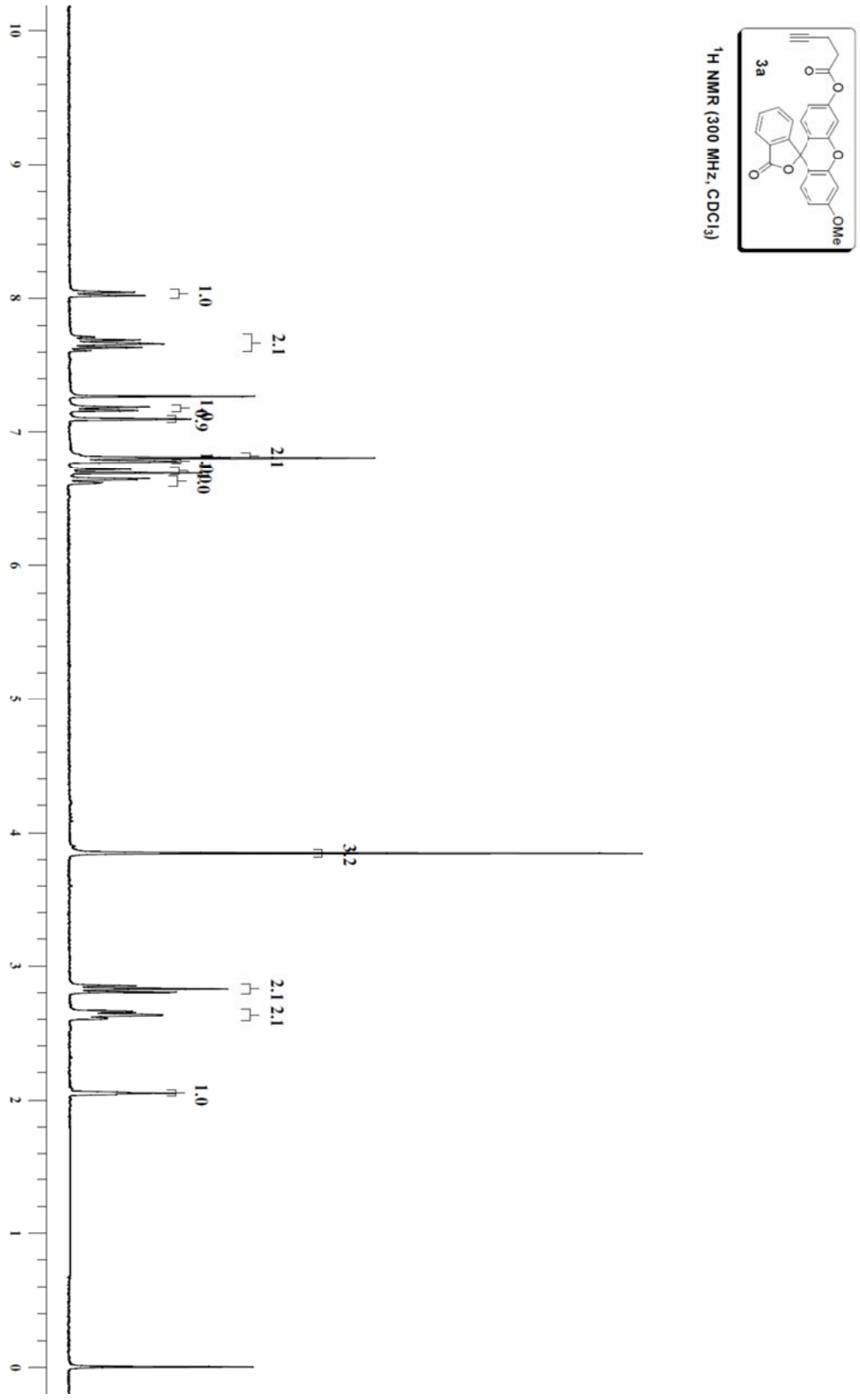
^{13}C NMR (75 MHz, $CDCl_3$): 169.1, 164.2, 153.0, 152.3, 152.0, 151.6, 151.5, 141.7, 135.3, 134.3, 133.5, 131.7, 131.6, 131.1, 130.0, 129.1, 128.7, 128.3, 128.1, 128.0, 125.9, 125.2, 124.0, 117.8, 116.8, 110.5, 94.7, 81.5.

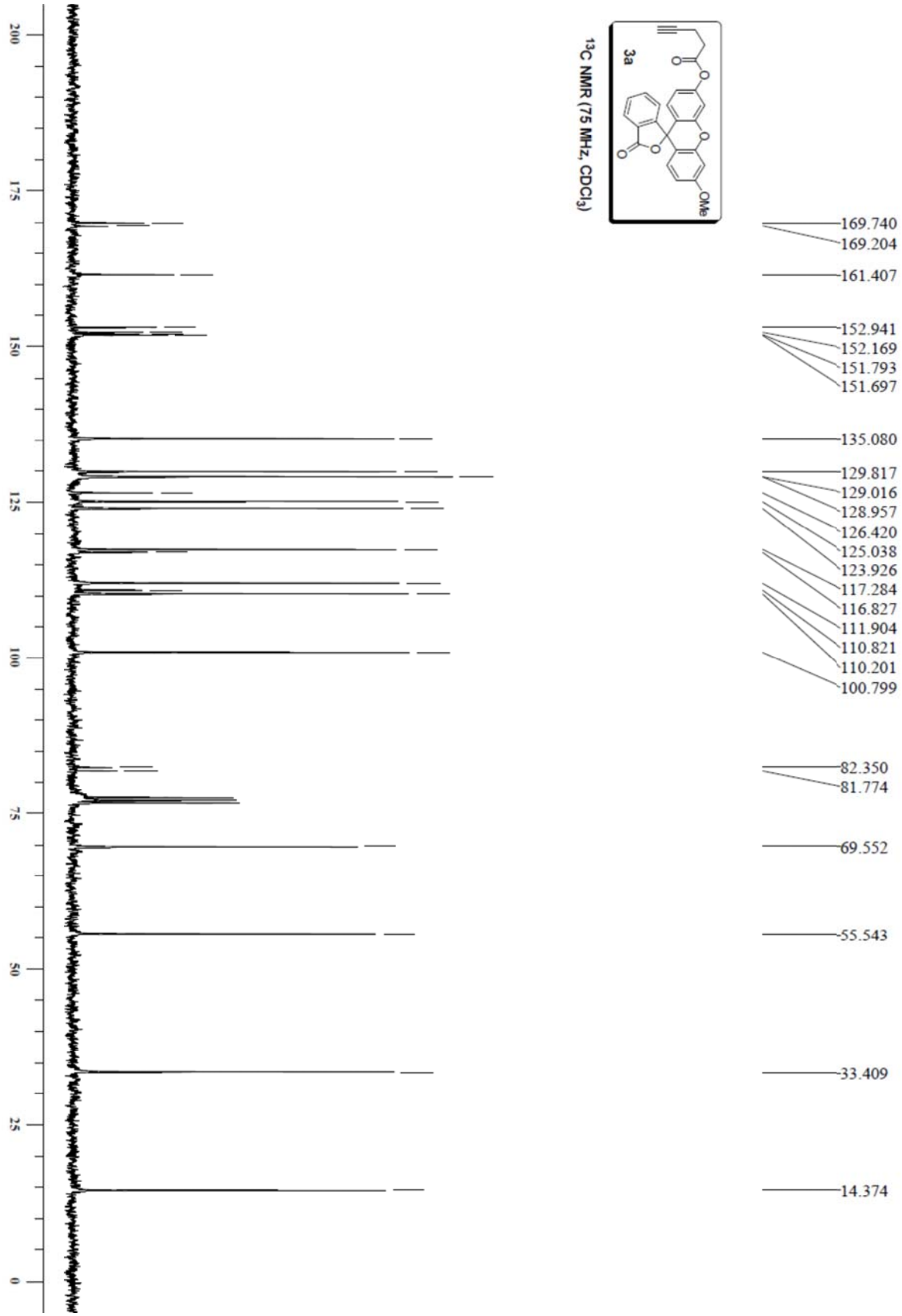
IR (film): ν_{max} 1749, 1610, 1582, 1492, 1422, 1234, 1152, 1012, 888, 738 cm^{-1}.

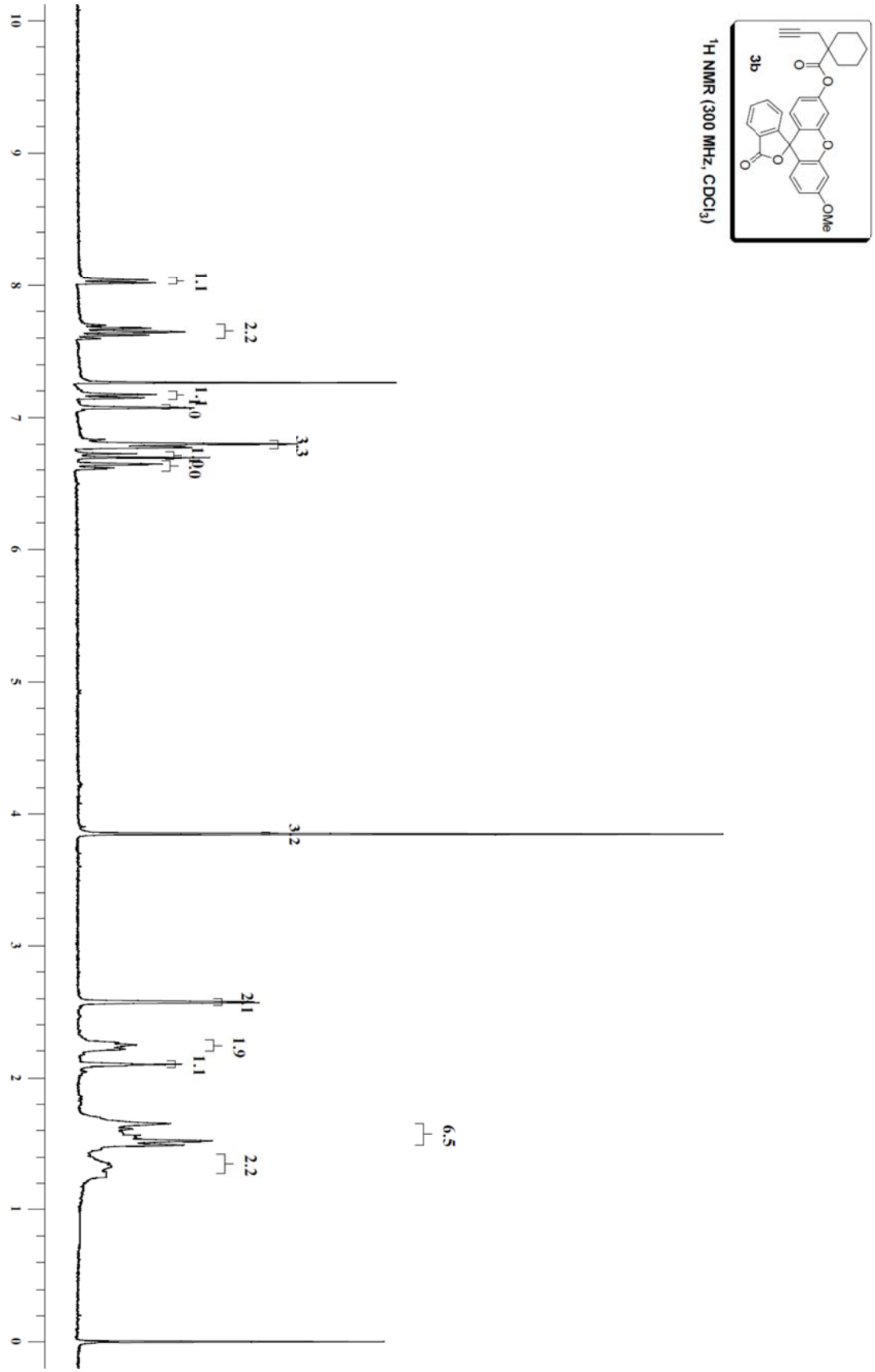
MS (ESI) *m/z* 741.0 (M^+ + H); HRMS calcd for $C_{50}H_{28}O_7$ (M^+ + H) 741.19133 found 741.19128.

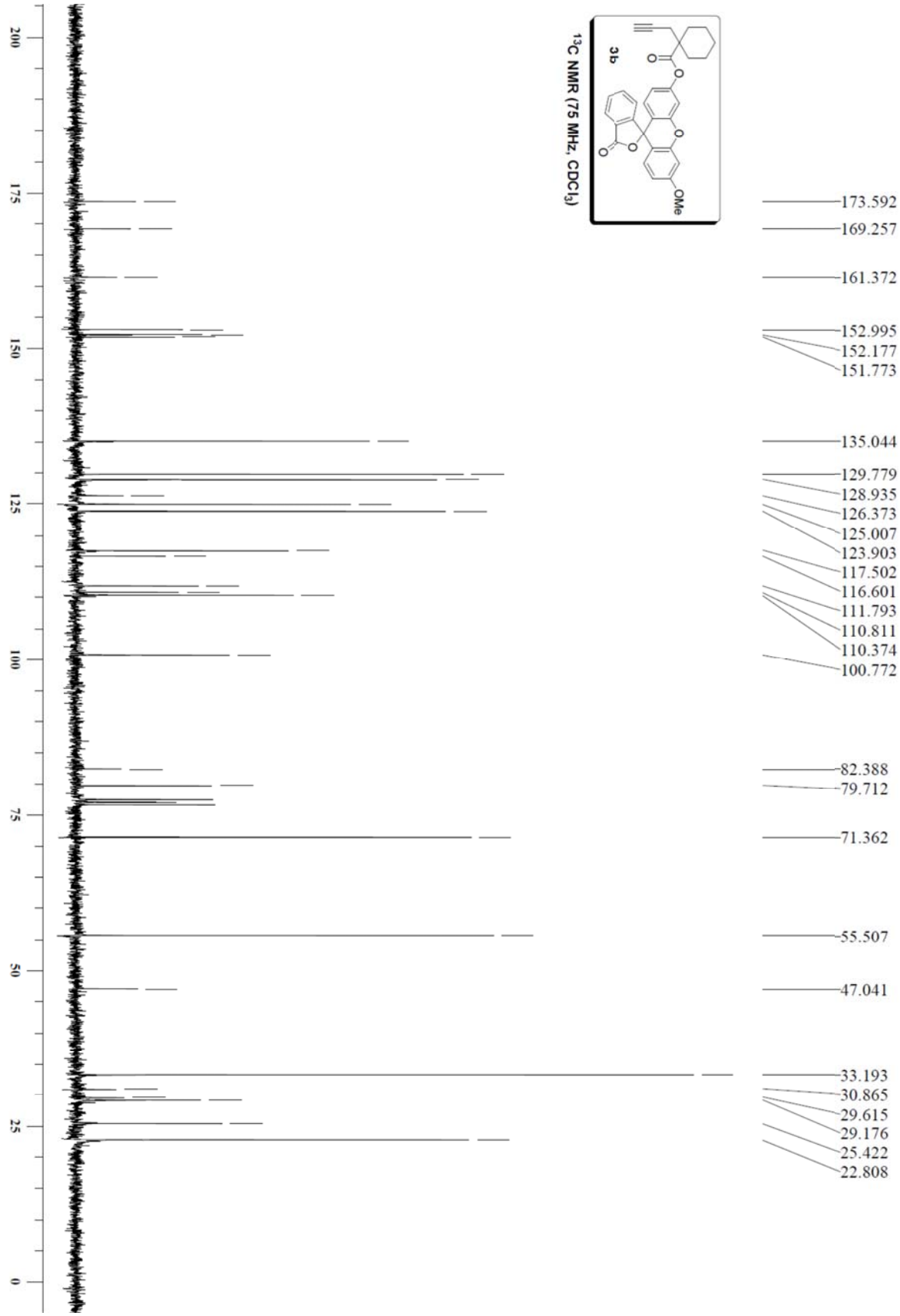
As suggested by referee, the time dependence studies were performed with the probe **C** and **D**. Indeed, probe **C** found to be good in respect of fluorescence response; however, probe **D** turned out to be less responsive. The time dependence studies are given in analytical section. However, the bioimaging studies with both the probes proved not to be satisfactory.

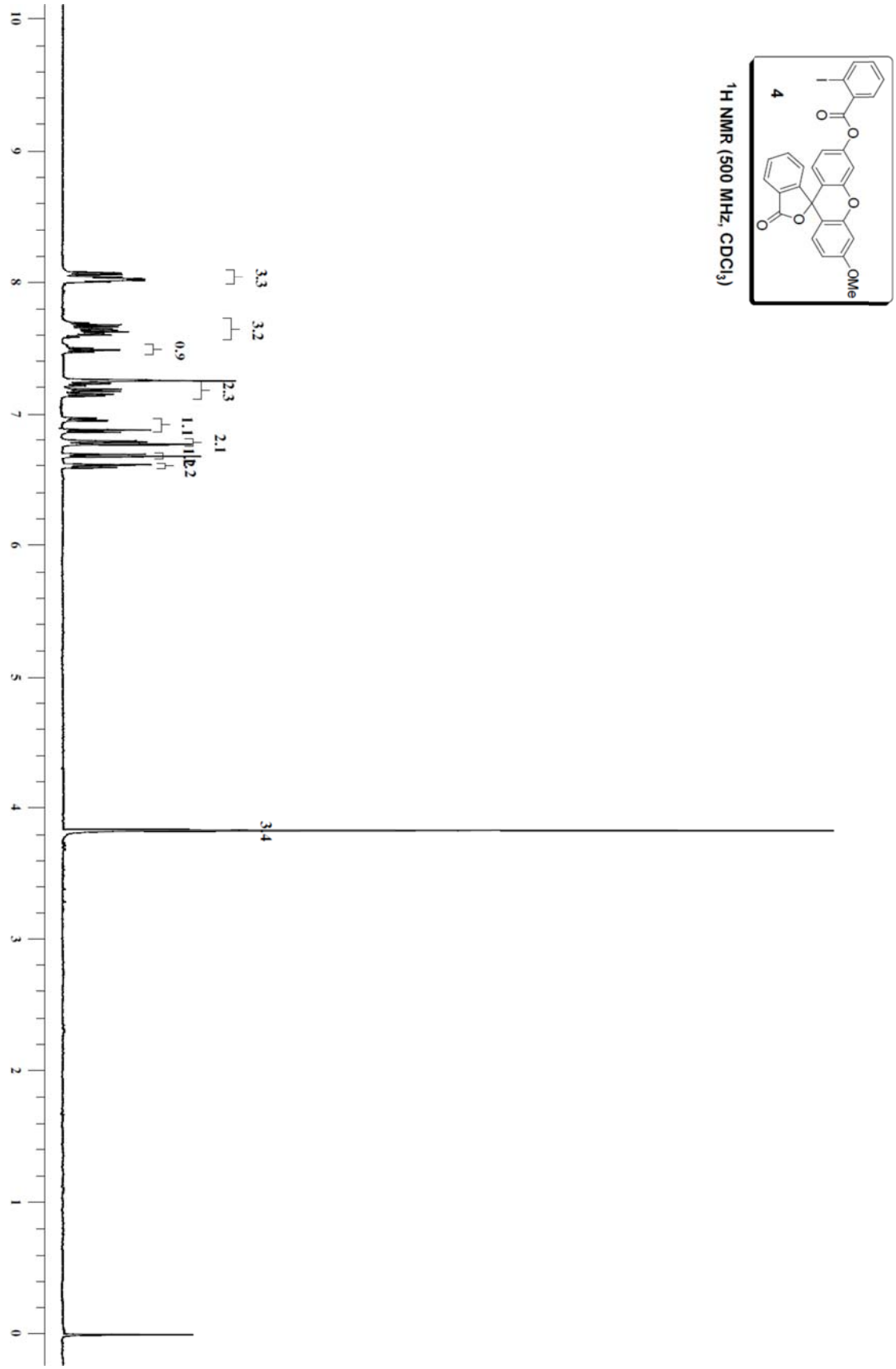
2.5 ^{1}H NMR and ^{13}C NMR Spectra of Compounds

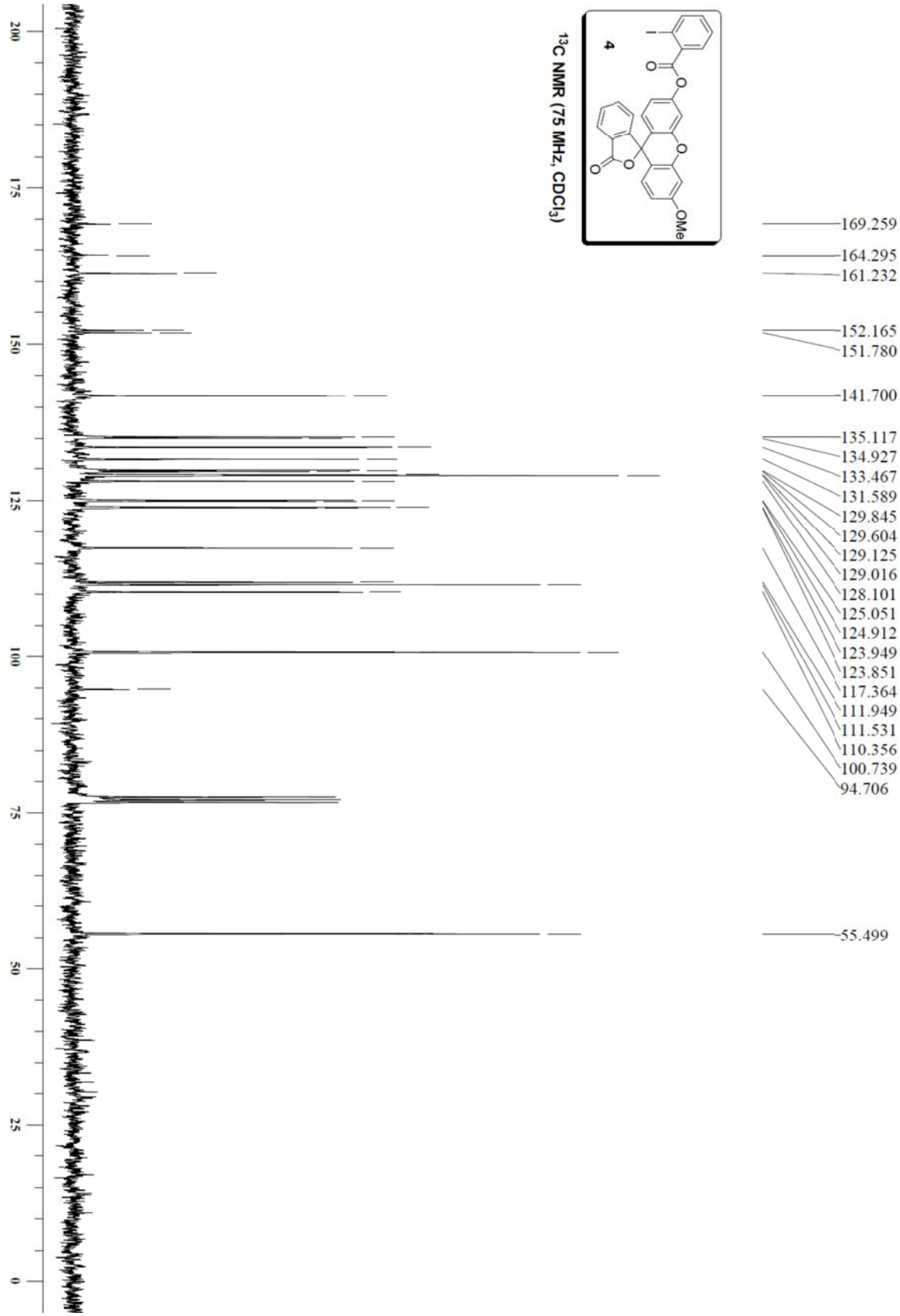


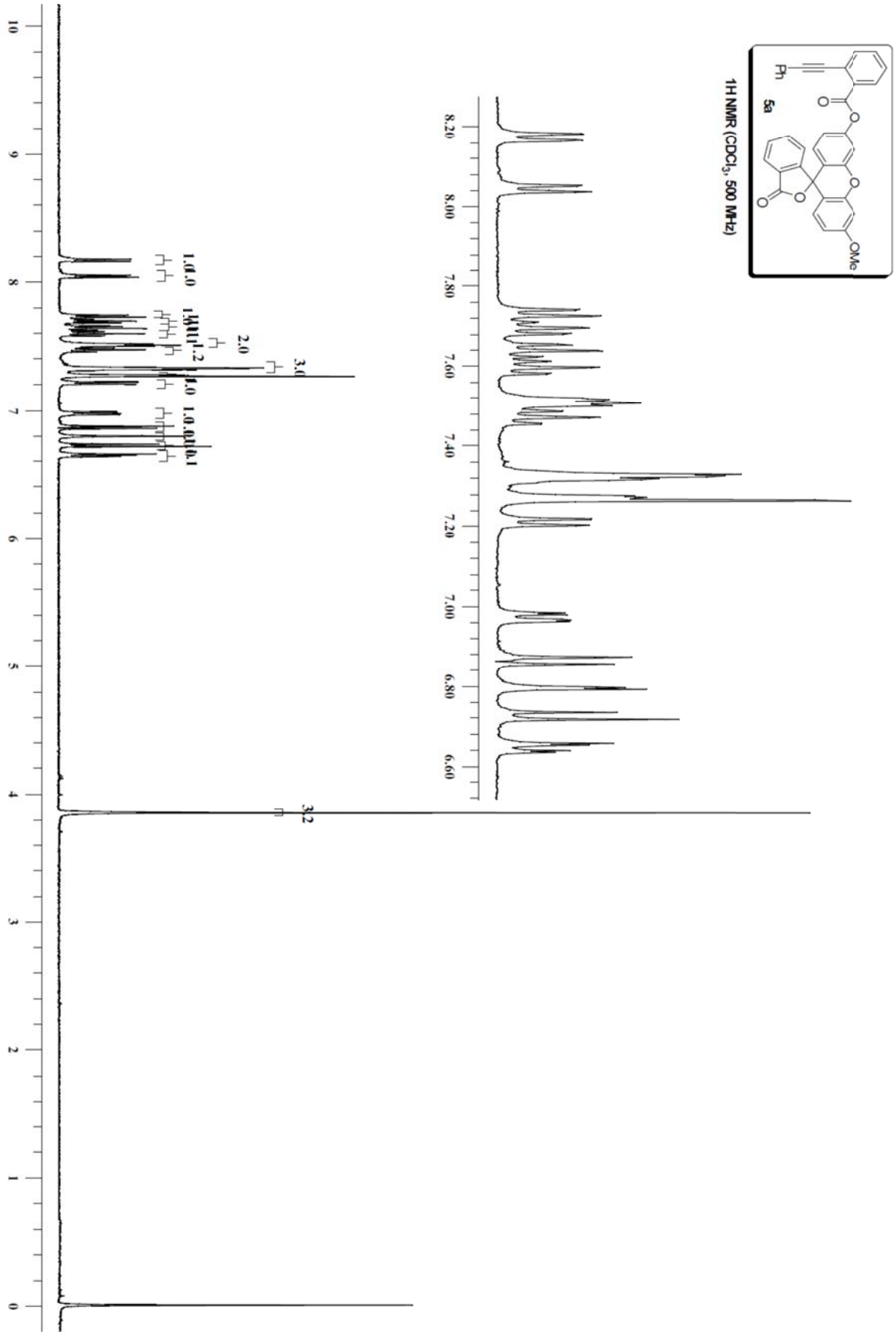

^{13}C NMR (75 MHz, CDCl$_3$)
3a
OMe
200
175
150
125
100
75
50
25
0
169.740
169.204
161.407
152.941
152.169
151.793
151.697
135.080
129.817
129.016
128.957
126.420
125.038
123.926
117.284
116.827
111.904
110.821
110.201
100.799
82.350
81.774
69.552
55.543
33.409
14.374

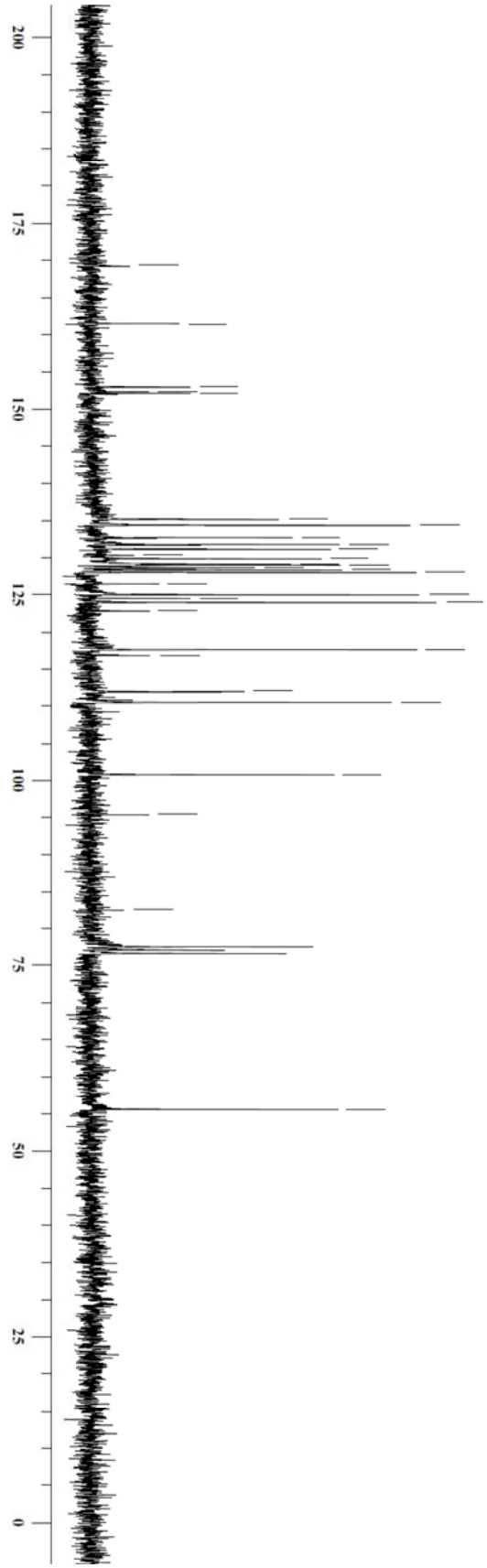

3b
OMe
^{1}H NMR (300 MHz, CDCl$_3$)
1.1
2.2
1.1
1.0
3.3
1.0
2.0
3.2
2.1
1.9
1.1
6.5
2.2


^{13}C NMR (75 MHz, CDCl$_3$)
3b
OMe
200
175
150
125
100
75
50
25
0
173.592
169.257
161.372
152.995
152.177
151.773
135.044
129.779
128.935
126.373
125.007
123.903
117.502
116.601
111.793
110.811
110.374
100.772
82.388
79.712
71.362
55.507
47.041
33.193
30.865
29.615
29.176
25.422
22.808

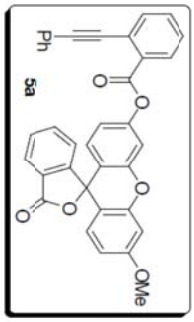

4
OMe
^{1}H NMR (500 MHz, CDCl$_3$)
3.3
3.2
0.9
2.3
2.1
1.1
2.2
3.4


^{13}C NMR (75 MHz, CDCl$_3$)
4
I
O
O
O
O
O
O
OMe
200
175
150
125
100
75
50
25
0
169.259
164.295
161.232
152.165
151.780
141.700
135.117
134.927
133.467
131.589
129.845
129.604
129.125
129.016
128.101
125.051
124.912
123.949
123.851
117.364
111.949
111.531
110.356
100.739
94.706
55.499

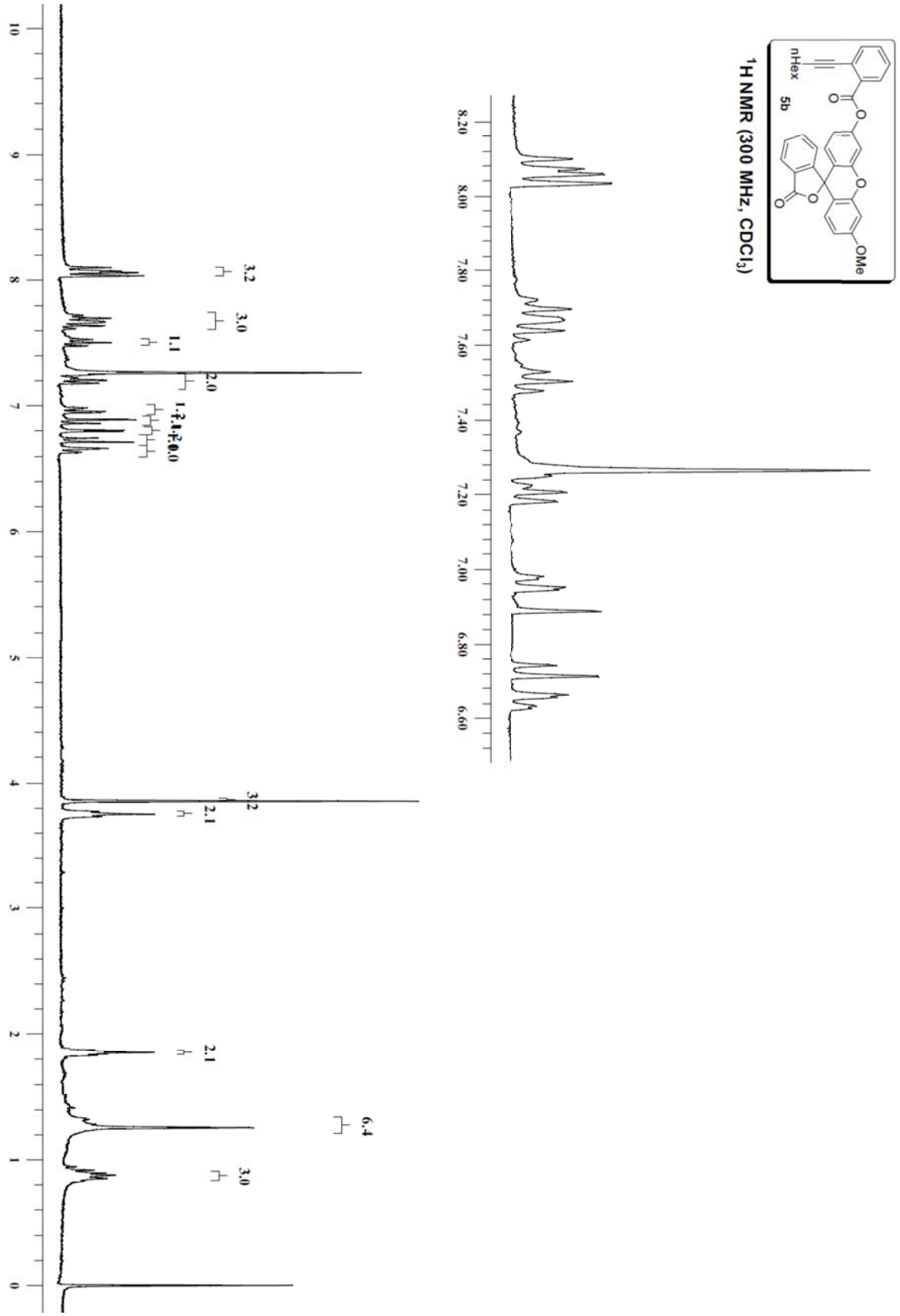

1H NMR (CDCl3, 500 MHz)
Ph
5a
OMe
8.20
8.00
7.80
7.60
7.40
7.20
7.00
6.80
6.60
3.2
2.0
1.0
1.2

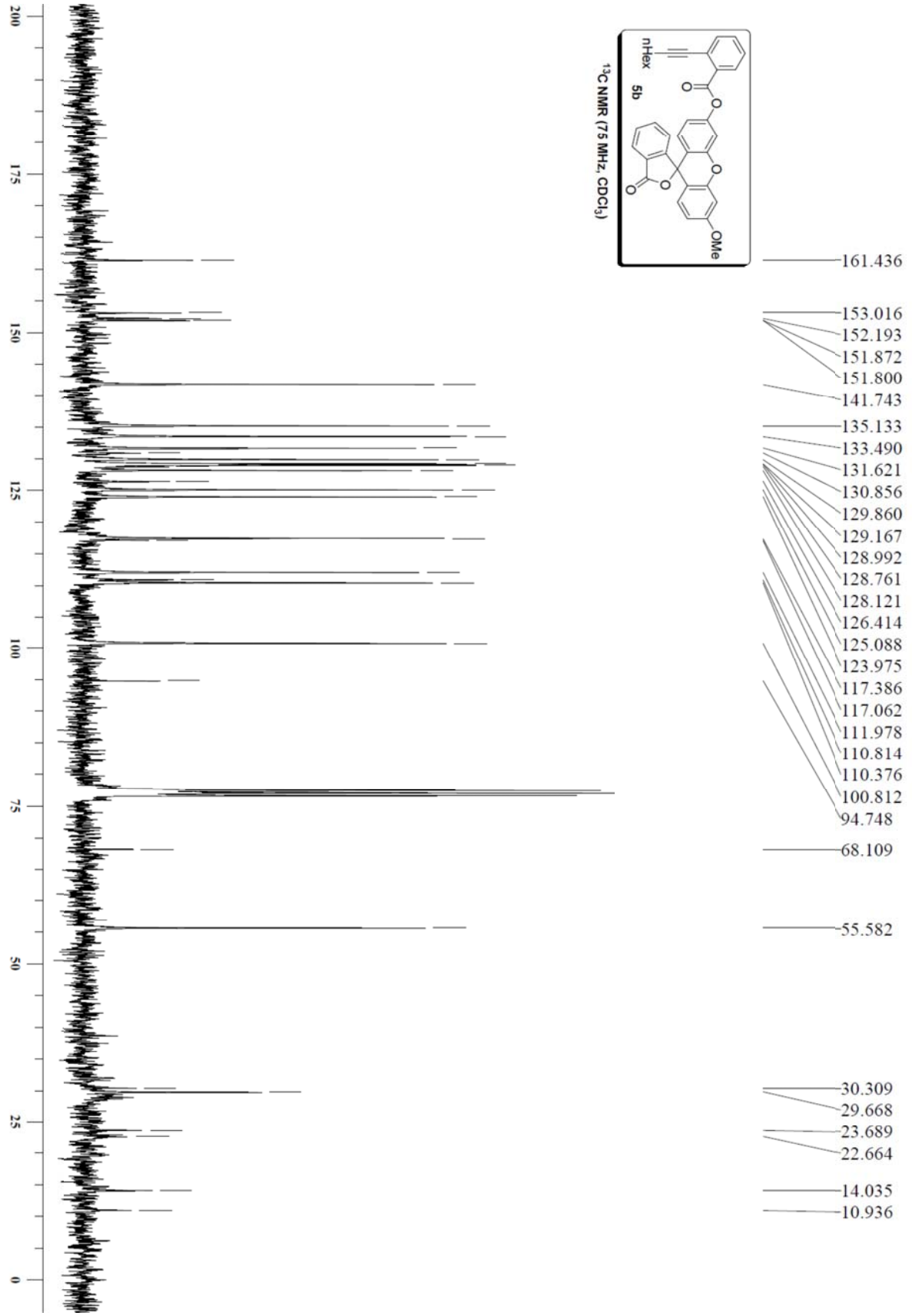


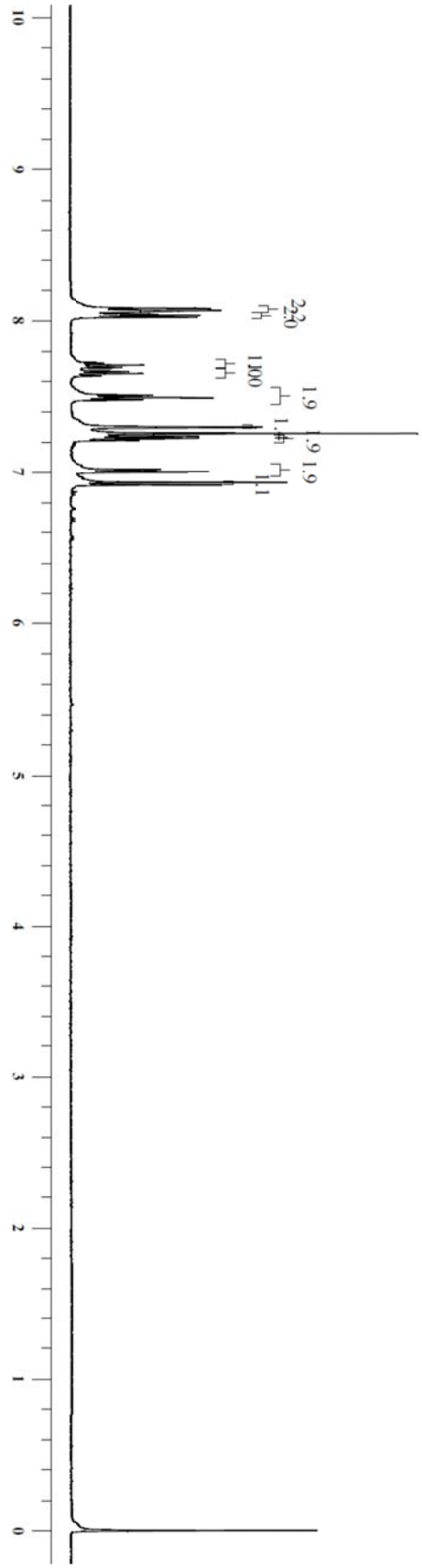
^{13}C NMR ($CDCl_3$, 75 MHz)

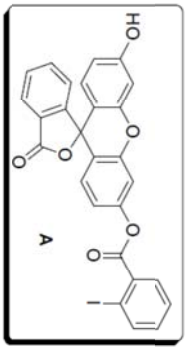


169.284
161.401
152.935
152.235
152.093
135.103
134.286
132.600
131.728
131.085
130.271
129.804
129.107
128.963
128.641
128.288
128.003
126.463
125.036
124.542
123.988
122.883
117.529
116.746
111.910
110.459
100.772
95.304
82.473
55.534

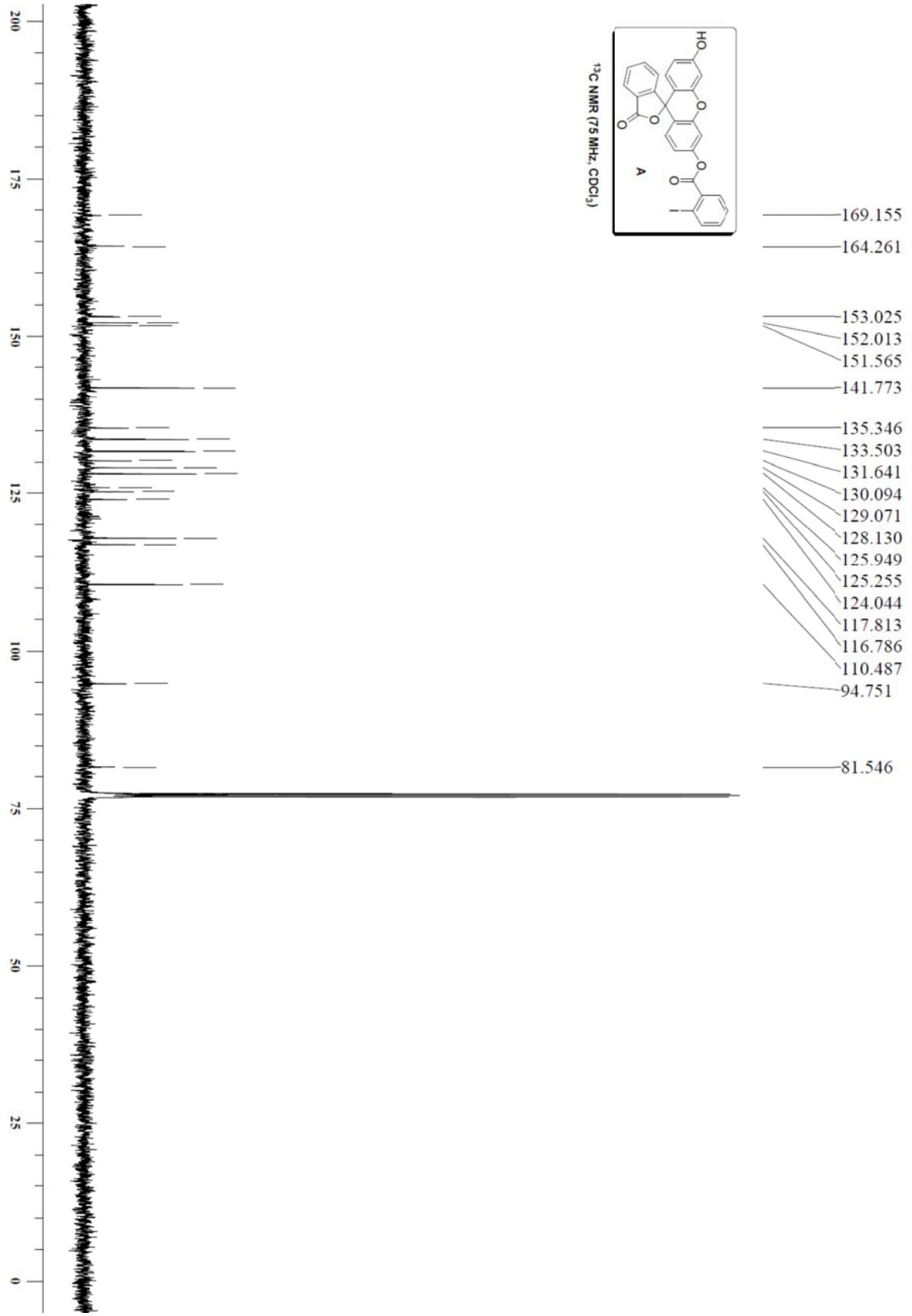

^{1}H NMR (300 MHz, CDCl$_3$)
nHex
5b
OMe
8.20
8.00
7.80
7.60
7.40
7.20
7.00
6.80
6.60
10
9
8
7
6
5
4
3
2
1
0
3.2
3.0
1.1
2.0
1.3
1.1
2.0
3.2
2.1
2.1
6.4
3.0

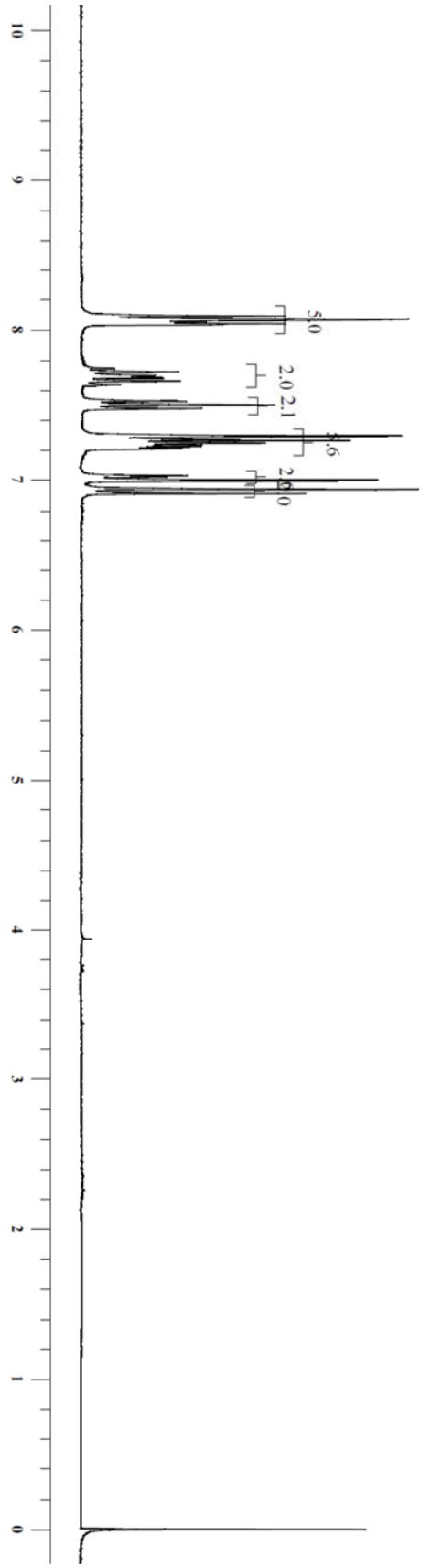

^{13}C NMR (75 MHz, CDCl$_3$)
nHex
5b
OMe
200
175
150
125
100
75
50
25
0
161.436
153.016
152.193
151.872
151.800
141.743
135.133
133.490
131.621
130.856
129.860
129.167
128.992
128.761
128.121
126.414
125.088
123.975
117.386
117.062
111.978
110.814
110.376
100.812
94.748
68.109
55.582
30.309
29.668
23.689
22.664
14.035
10.936



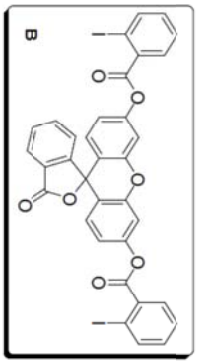


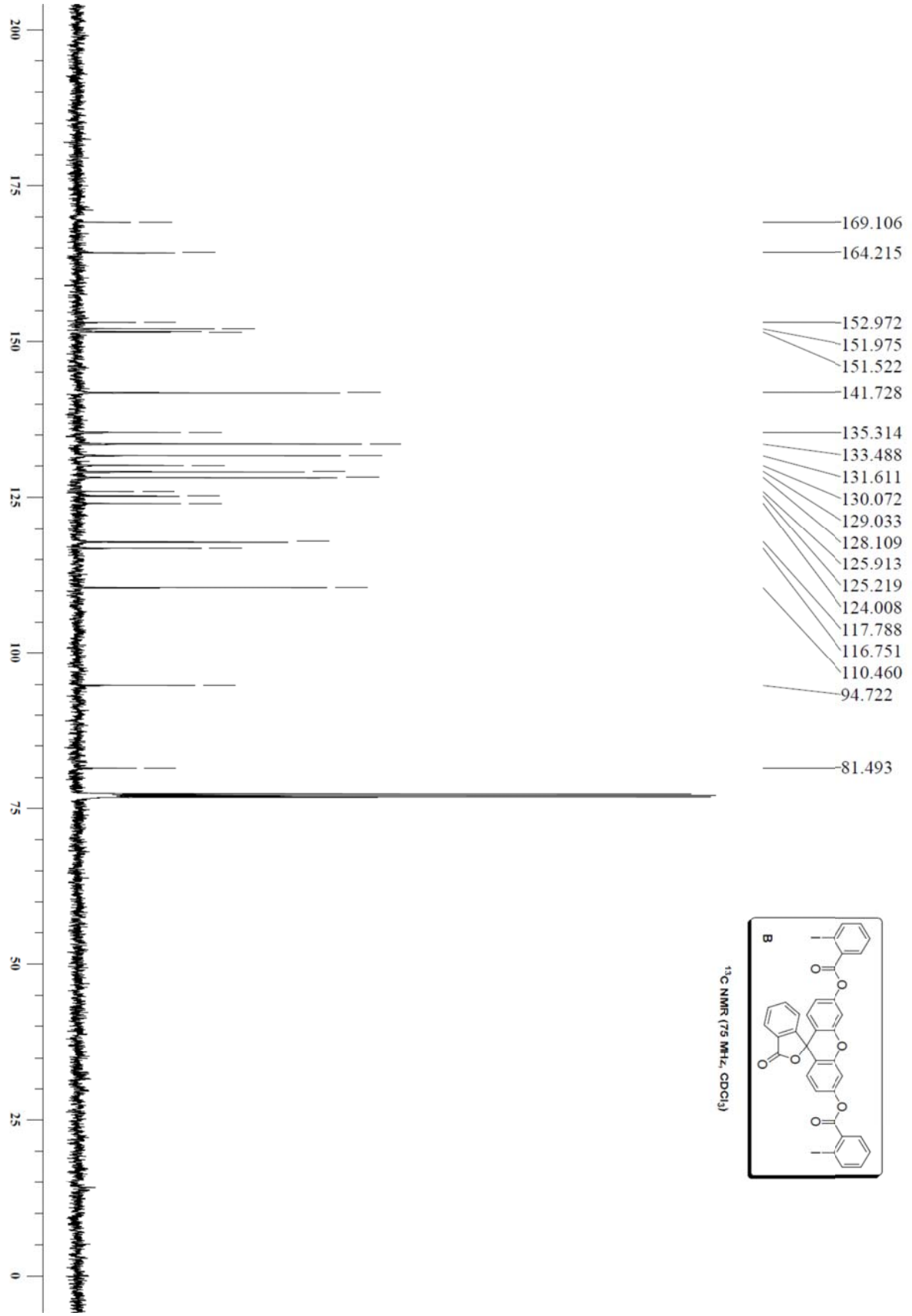
^{1}H NMR (300MHz, $CDCl_3$)

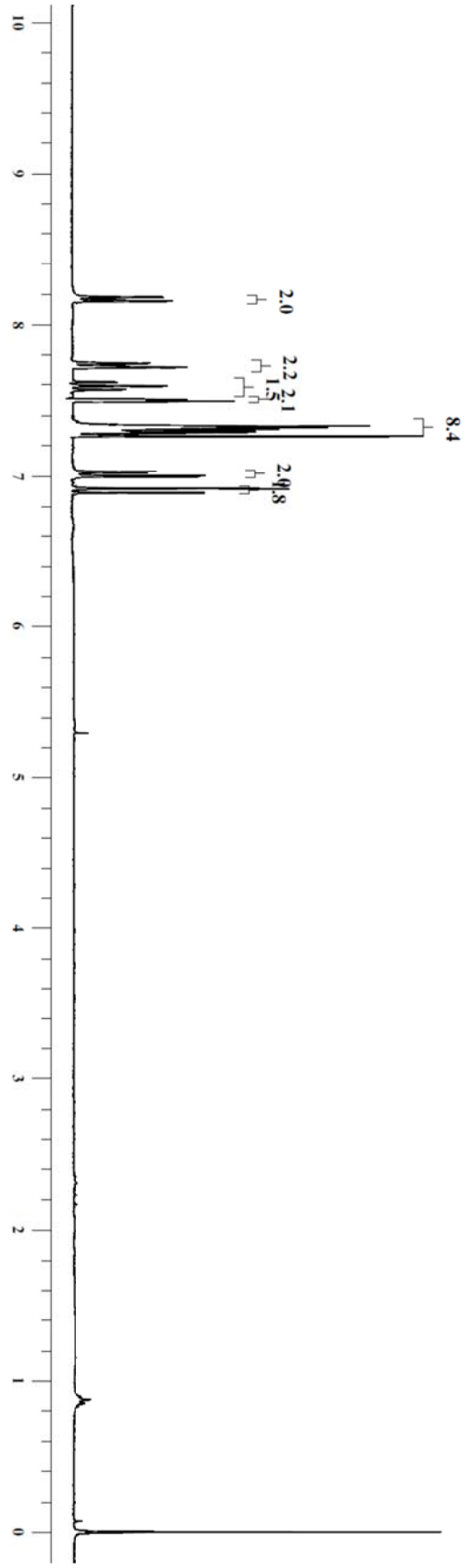

^{13}C NMR (75 MHz, CDCl$_3$)
HO
O
O
O
O
O
I
A
200
175
150
125
100
75
50
25
0
169.155
164.261
153.025
152.013
151.565
141.773
135.346
133.503
131.641
130.094
129.071
128.130
125.949
125.255
124.044
117.813
116.786
110.487
94.751
81.546



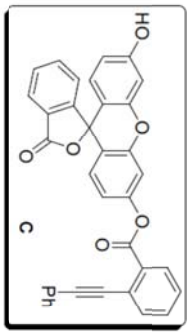
^{1}H NMR (300 MHz, $CDCl_3$)



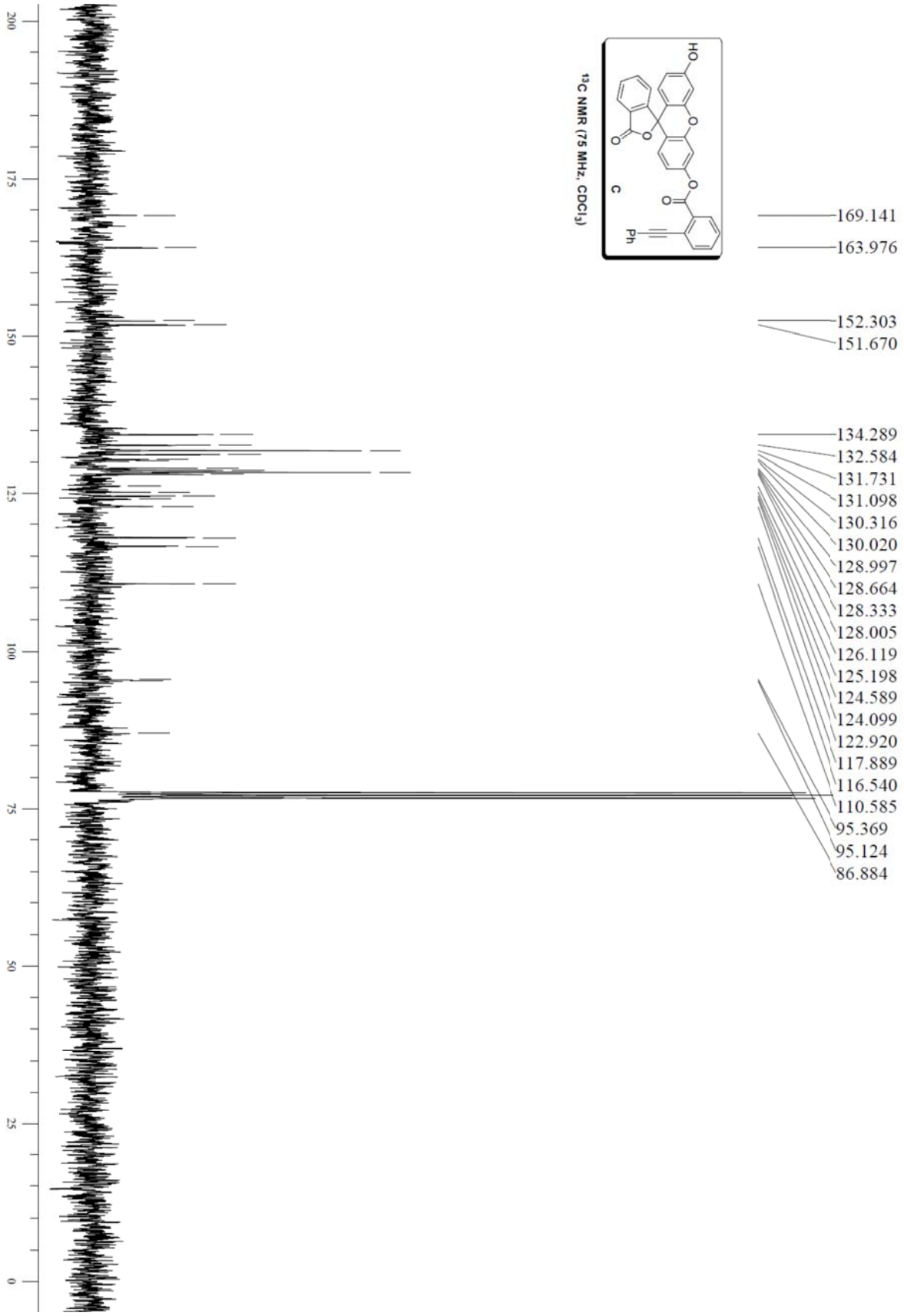

200
175
150
125
100
75
50
25
0
169.106
164.215
152.972
151.975
151.522
141.728
135.314
133.488
131.611
130.072
129.033
128.109
125.913
125.219
124.008
117.788
116.751
110.460
94.722
81.493
B
^{13}C NMR (75 MHz, CDCl$_3$)

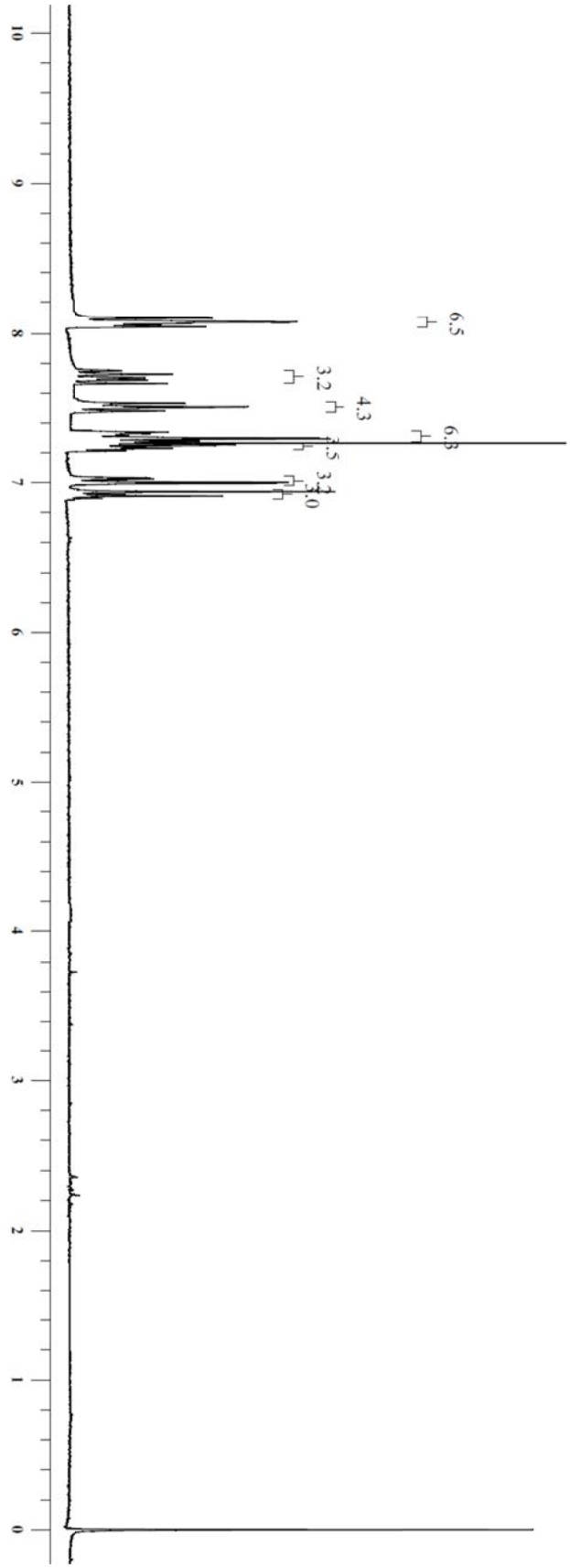


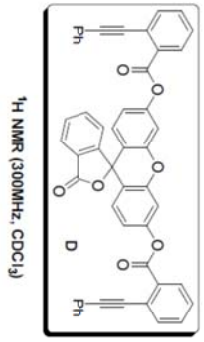
^{1}H NMR (300MHz, $CDCl_3$)



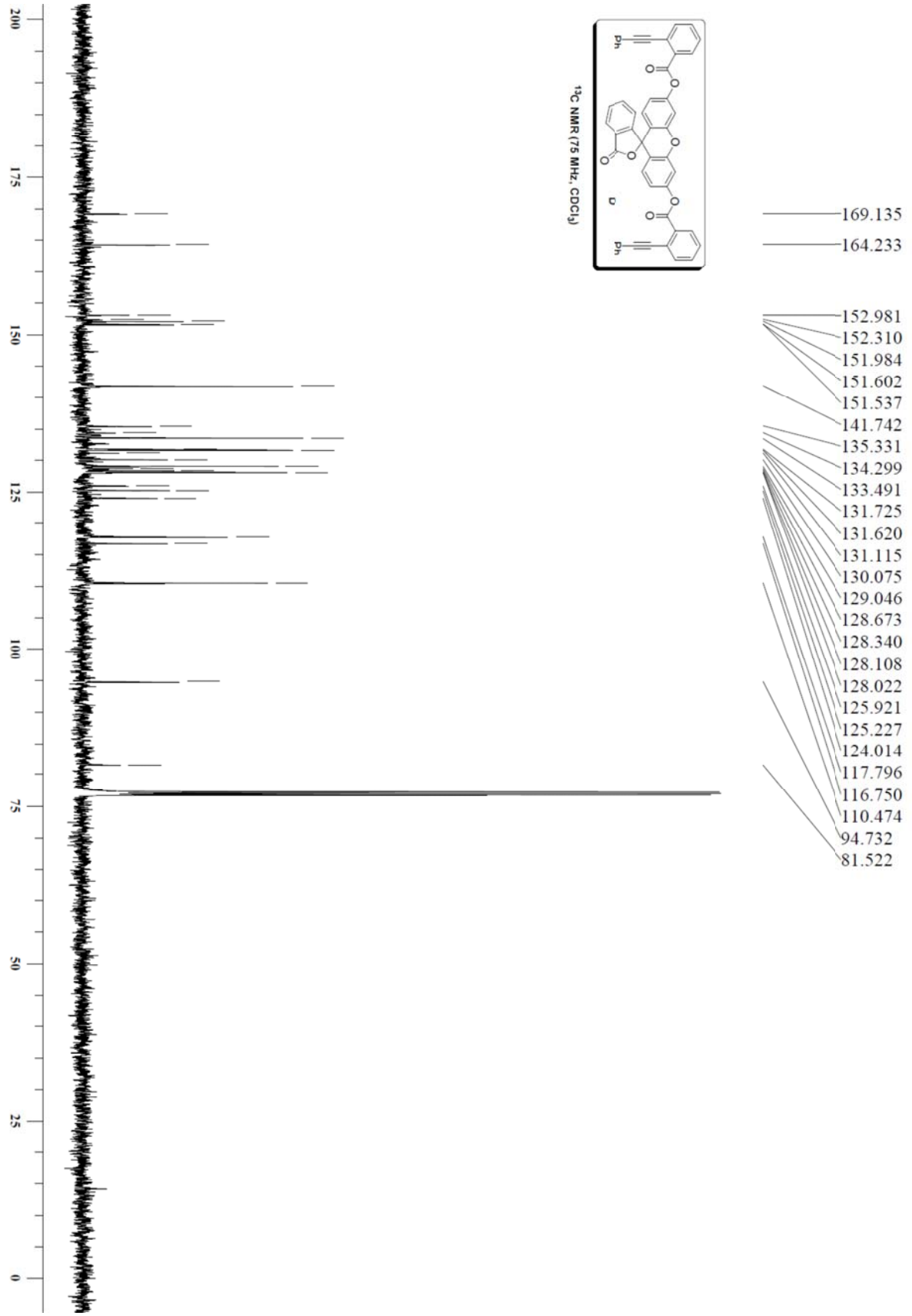
c


^{13}C NMR (75 MHz, CDCl$_3$)
HO
O
O
O
O
O
C
Ph
169.141
163.976
152.303
151.670
134.289
132.584
131.731
131.098
130.316
130.020
128.997
128.664
128.333
128.005
126.119
125.198
124.589
124.099
122.920
117.889
116.540
110.585
95.369
95.124
86.884
200
175
150
125
100
75
50
25
0





^{1}H NMR (300MHz, $CDCl_3$)


^{13}C NMR (75 MHz, CDCl$_3$)
169.135
164.233
152.981
152.310
151.984
151.602
151.537
141.742
135.331
134.299
133.491
131.725
131.620
131.115
130.075
129.046
128.673
128.340
128.108
128.022
125.921
125.227
124.014
117.796
116.750
110.474
94.732
81.522
200
175
150
125
100
75
50
25
0

3. Analytical Section:

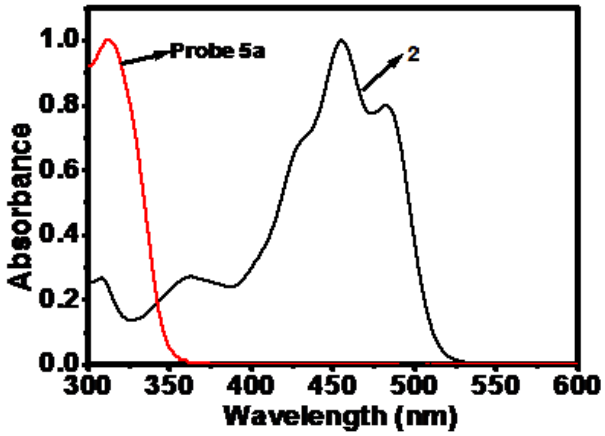


Figure1. Normalized absorption spectrum of probe **5a** and the fluorescein derivative **2**.

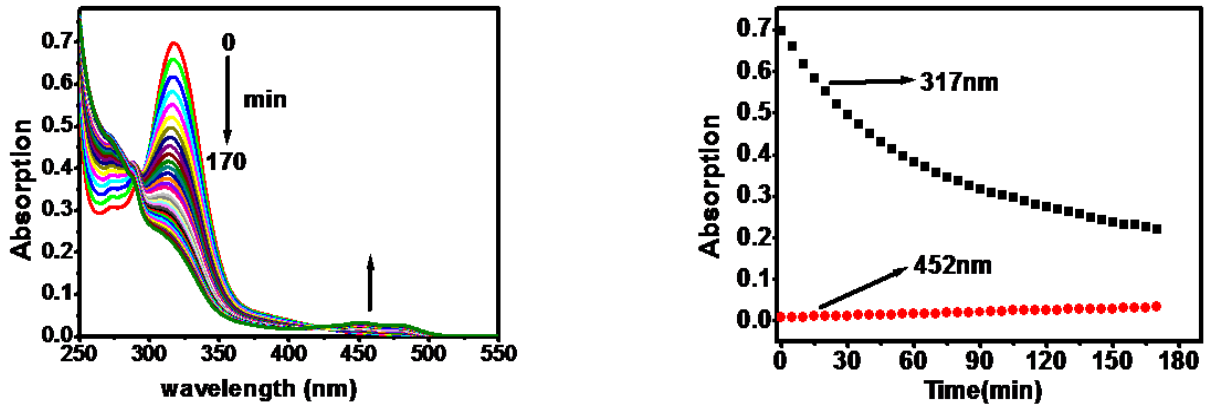


Figure 2. Time dependent absorption change and its kinetics upon addition of 10 equivalents of Au^{+3} to **5a** (10 μM) in CH_3CN:PBS buffer (1:1,0.1M, pH = 7.4). Isosbestic point at 424 nm

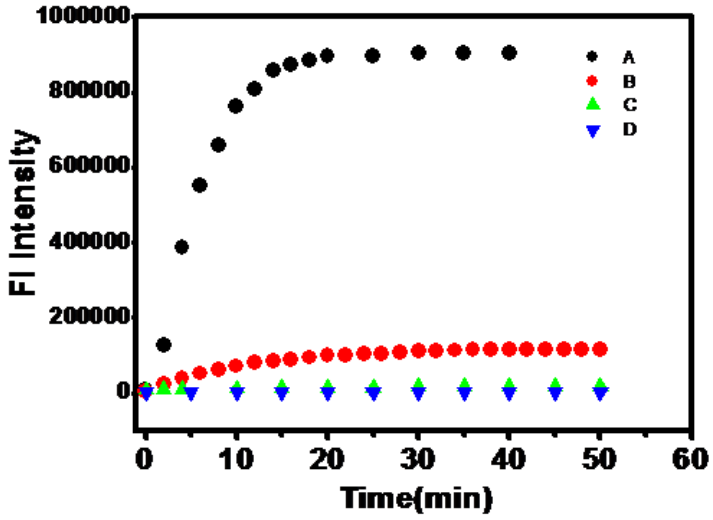


Figure 3. Fluorescence response of probes: Plot of fluorescence intensity at 515 nm against time for (A) Probe **5a**, (B) Probe **5b**, (C) Probe **3a**, (D) Probe **3b** (all Probes-10 μM) in the presence of Au^{1+} (100 μM) in CH_3CN:PBS buffer (1:1, 0.1M, pH = 7.4). λ_{excit} = 452 nm.

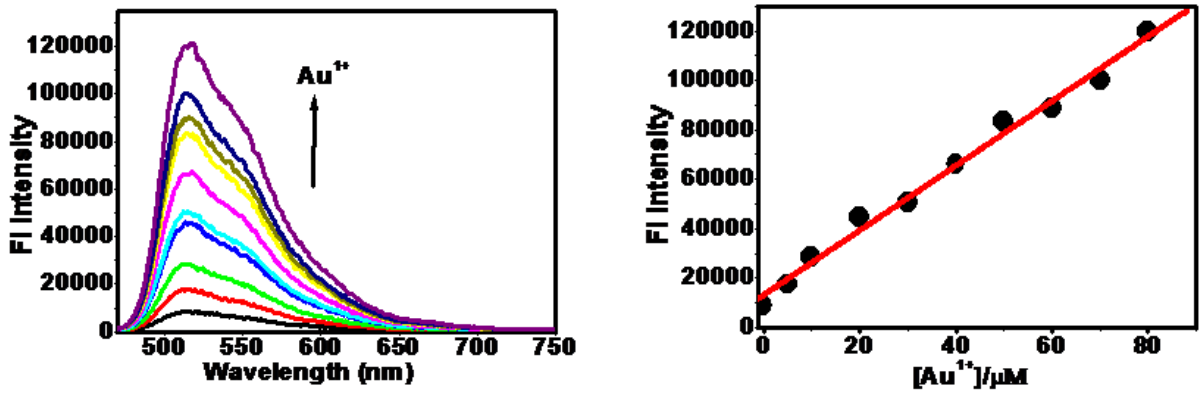


Figure 4. (a) Concentration dependent fluorescence change acquired for a mixture of **5a** (10 μM) upon addition of Au^{1+} (5 μM-80 μM) in CH_3CN:PBS buffer (1:1,0.1M, pH = 7.4). λ_{excit} = 452 nm. (b) Plot of fluorescence intensity at 515nm against Au^{1+} concentration. Each spectrum is taken after 2 min of Au^{1+} addition.

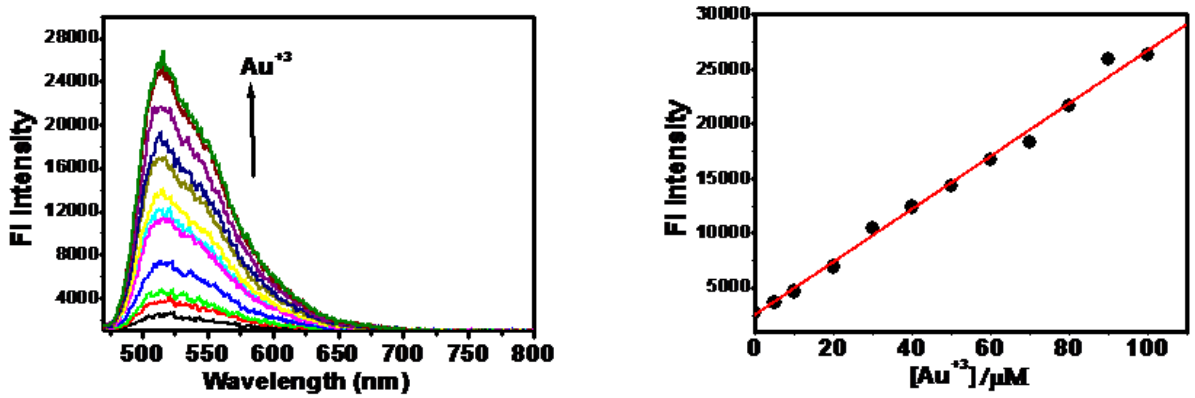


Figure 5. Concentration dependent fluorescence change acquired for a mixture of 1 (10 μM) and Au^{3+} (100 μM) in CH_3CN:PBS buffer (1:1, pH = 7.4). λ_{excit} = 452 nm. (b) Plot of fluorescence intensity at 515nm against Au^{+3} concentrations. Each spectrum is taken after 5 min of Au^{3+} additions.

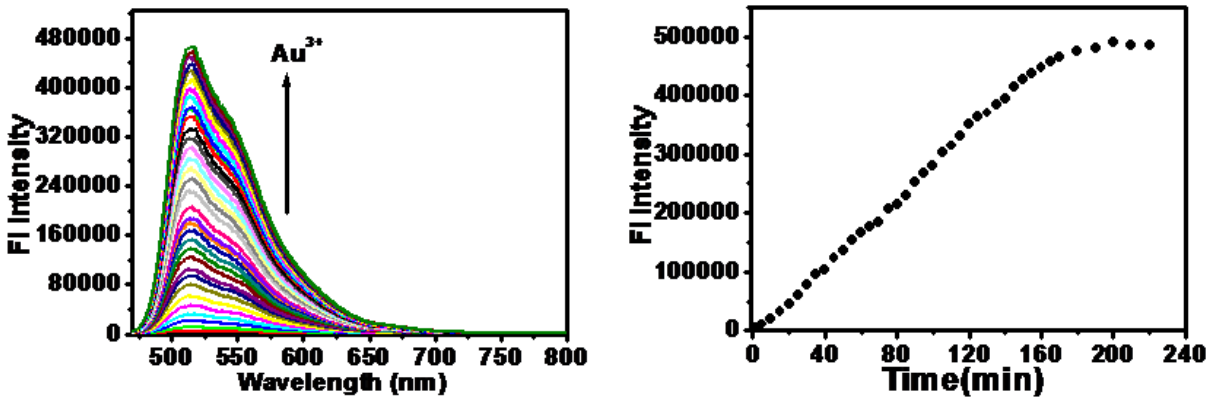


Figure 6. (a)Time dependent fluorescence change obtained for a mixture of 5a (10 μM) and Au^{3+} (100 μM) in CH_3CN:PBS buffer (1:1, 0.1M, pH = 7.4). λ_{excit} = 452 nm. (b) Plot of fluorescence intensity at 515nm against time.

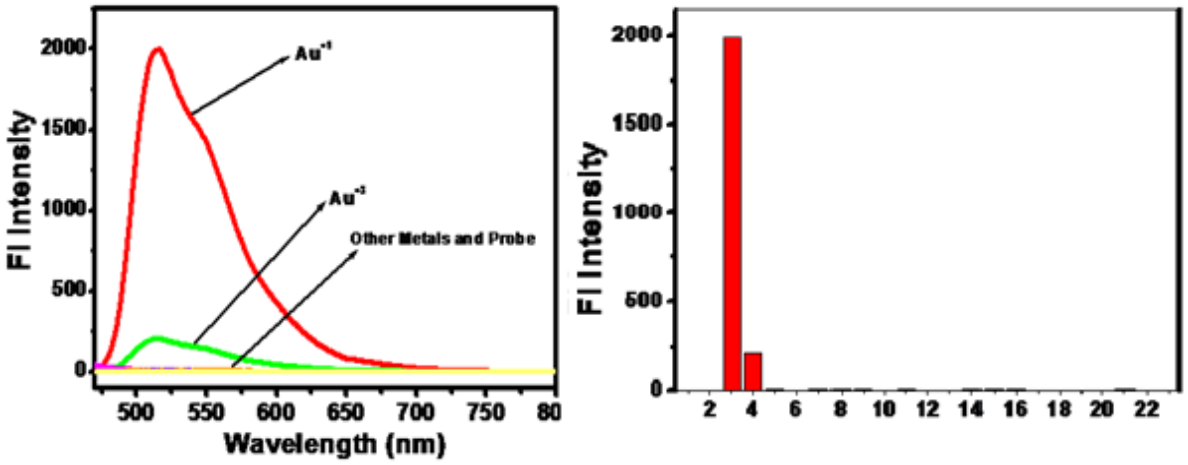


Figure 7. Fluorescence response of **5a** (10 μM) in the presence of various metals ions (100 μM). Excitation at 452 nm and emission monitoring at 515 nm. Metal ions: 1) probe **5a**, 2) Ag^{1+}, 3) Au^{1+}, 4) Au^{3+}, 5) Ba^{2+}, 6) Fe^{3+}, 7) Bi^{3+}, 8) Hg^{2+}, 9) Ir^{3+}, 10) K^{1+}, 11) La^{3+}, 12) Mn^{2+}, 13) Ni^{2+}, 14) Pd^{2+}, 15) Pt^{2+}, 16) Pt^{4+}, 17) Ru^{3+}, 18) Sc^{3+}, 19) Yb^{3+}, 20) Zn^{2+}, 21) Cd^{2+}, 22) Cr^{3+}

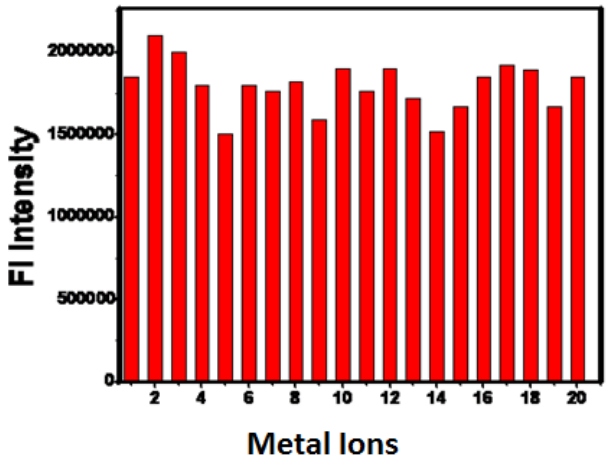


Figure 8. Fluorescence response of 1(10 μM) in the presence of Au^{1+} (100 μM) and other metals ions (100 μM). Excitation at 452 nm and emission monitoring at 515 nm. Metals: 1) Ag^{1+}, 2) Au^{1+}, 3) Au^{3+}, 4) Ba^{2+}, 5) Fe^{3+}, 6) Bi^{3+}, 7) Hg^{2+}, 8) Ir^{3+}, 9) K^{+1}, 10) La^{3+}, 11) Mn^{2+}, 12) Ni^{2+}, 13) Pd^{2+}, 14) Pt^{2+}, 15) Pt^{4+}, 16) Ru^{3+}, 17) Sc^{3+}, 18) Yb^{3+}, 19) Zn^{2+}, 20) Cd^{2+}, 21) Cr^{3+}. Each spectrum is taken after 30 min of metal ion addition.

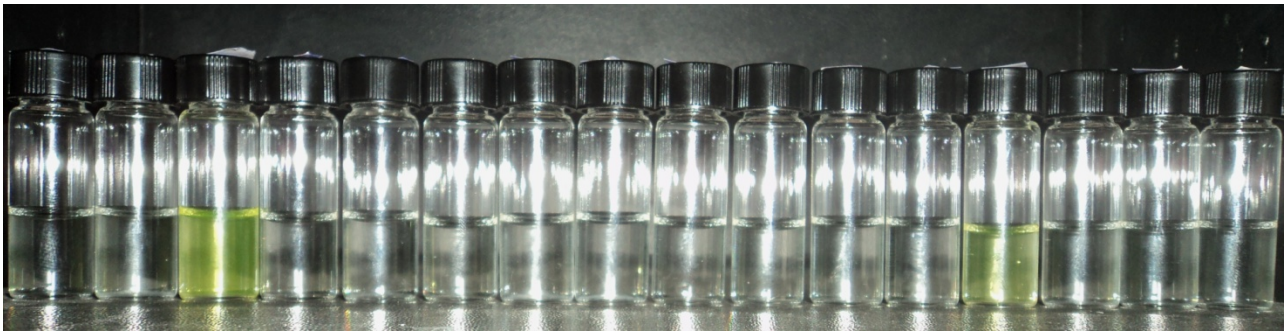


Figure 9. Naked eye detection of metal ions of **5a** (50 μM), Au^{1+} and Au^{3+} 10 μM, ions were added whereas100 μM of other ions were added. From left to right, no metal ion, Pt^{2+}, Au^{1+}, Hg^{2+}, Ag^{1+}, Mn^{2+}, Pd^{2+}, Ni^{2+}, Sc^{3+} $Cd^{2+,}$ Ru^{3+}, Ba^{2+}, Au^{3}+, Zn^{2+}, Fe^{3+}, Cr^{3+}.

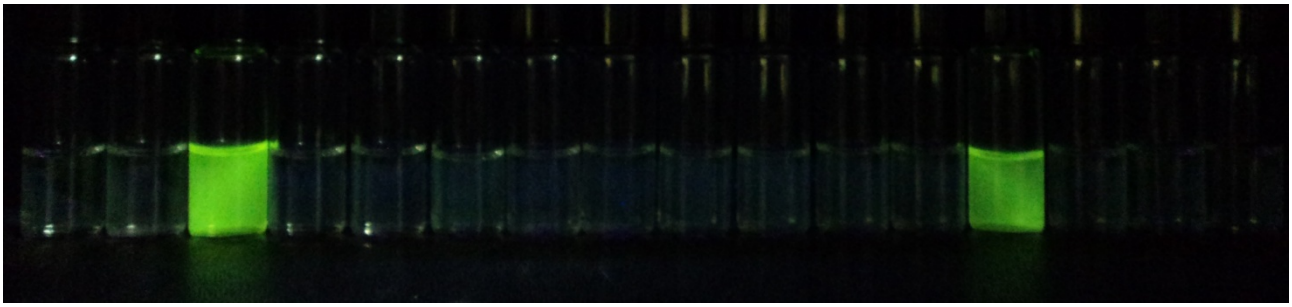


Figure 10. Fluorescence photographs of probe **5a** (50 μM), Au^{1+} and Au^{3+} 10 μM whereas 100 μM of different metal ions in CH_3CN / PBS buffer (1:1, v/v, pH = 7.4) recorded after 1 h at 25°C. From left to right, no metal ion, Pt^{2+}, Au^{1+}, Hg^{2+}, Ag^{1+}, Mn^{2+}, Pd^{2+}, Ni^{2+}, Sc^{3+} $Cd^{2+,}$ Ru^{3+}, Ba^{2+}, Au^{3}+, Zn^{2+}, Fe^{3+}, Cr^{3+}.

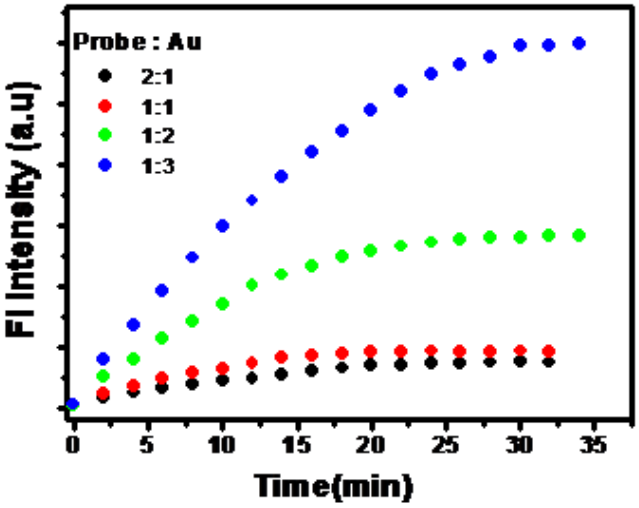


Figure 11. Fluorescence response of probe **5a** against different stoichiometric amounts of Au^{1+}. Plot of fluorescence intensity is measured at 515 nm against time. 10 μM of probe **5a** is used in all the experiments in CH_3CN: PBS buffer (1:1, 0.1M, pH = 7.4). (λ_{excit} = 452 nm).

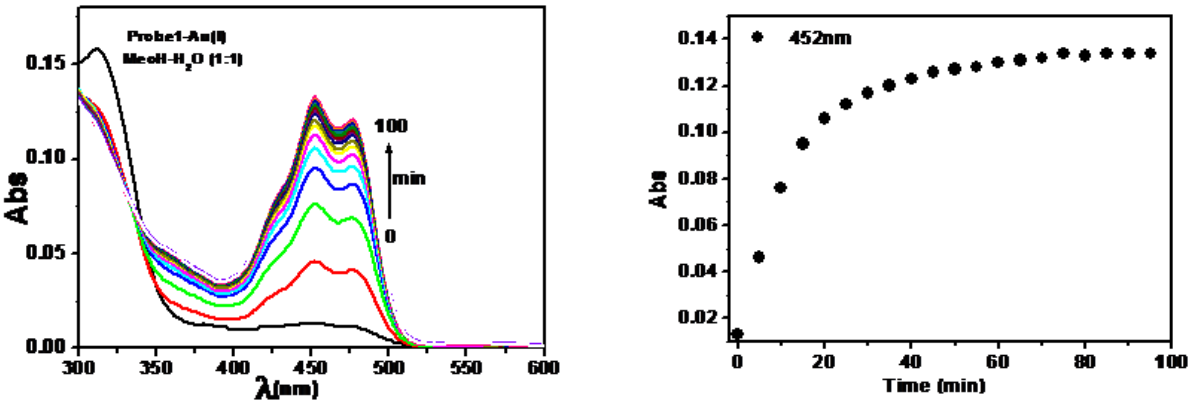


Figure 12. Time dependent absorption change and its kinetics upon addition of Au^{1+} (100 μM) to **5a** (10 μM) in MeOH:PBS buffer (1:1,0.1M, pH = 7.4). Isosbestic point at 424 nm.

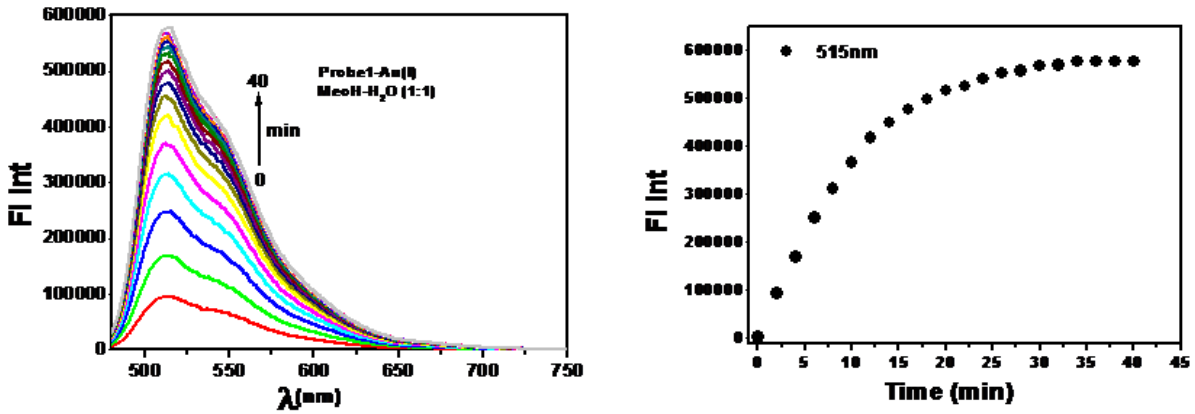


Figure 13. (a)Time dependent fluorescence change obtained for a mixture of **5a** (100 μM) and Au^{1+} (100 μM) in MeOH:PBS buffer (1:1, 0.1M, pH = 7.4). λ_{excit} = 452 nm. (b) Plot of fluorescence intensity at 515 nm against time.

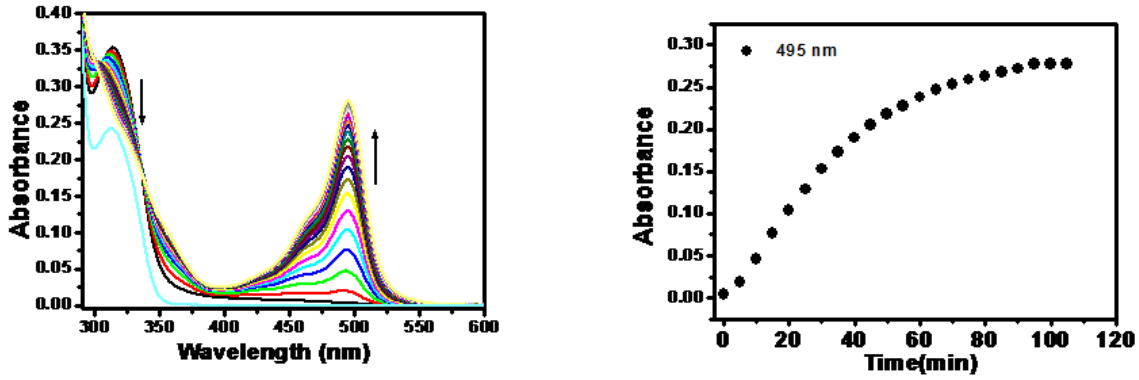


Figure 14. Time dependent absorption change and its kinetics upon addition of 10 equivalents of Au^{1+} to probe **C** (10 μM) in CH_3CN:PBS buffer (1:1, 0.1M, pH = 7.4).

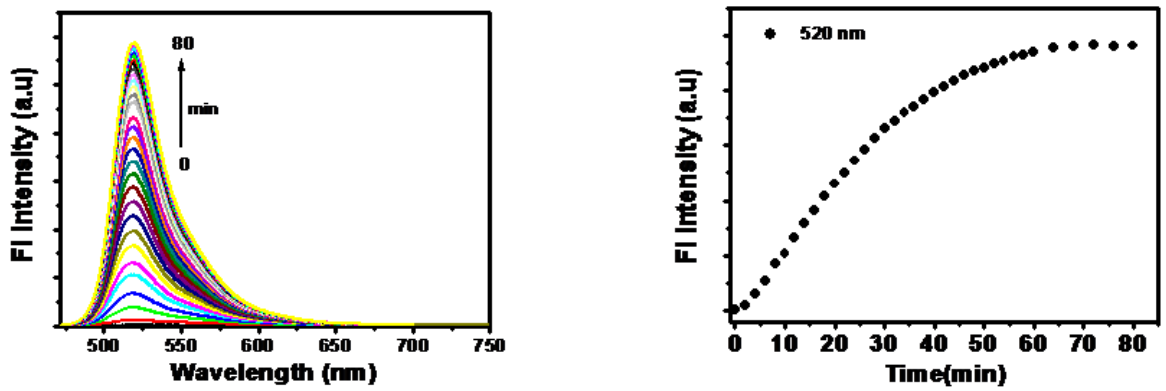


Figure 15. (a) Time dependent fluorescence change obtained for a mixture of probe **C** (10 μM) and Au^{1+} (100 μM) in CH_3CN:PBS buffer (1:1, 0.1M, pH = 7.4). λ_{excit} = 452 nm. (b) Plot of fluorescence intensity at 519 nm against time.

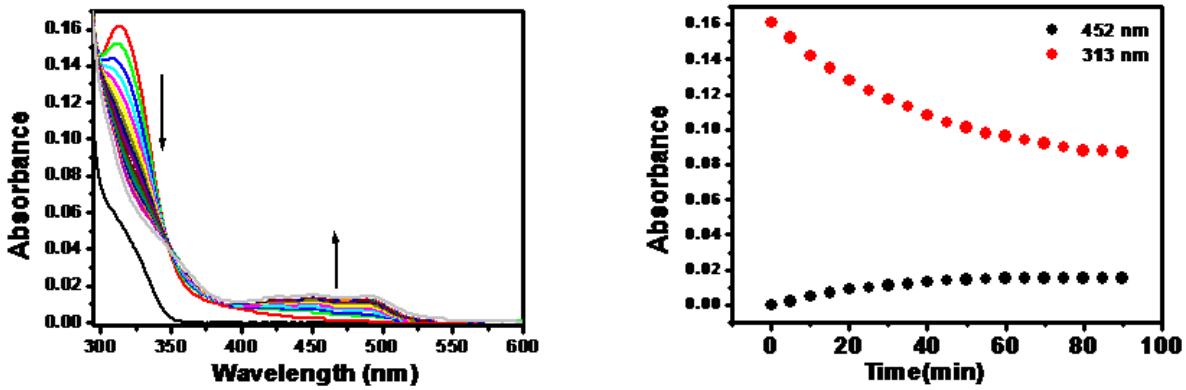


Figure 16. Time dependent absorption change and its kinetics upon addition of 10 equivalents of Au^{1+} to Probe **D** (10 μM) in CH_3CN:PBS buffer (1:1, 0.1M, pH-7.4).

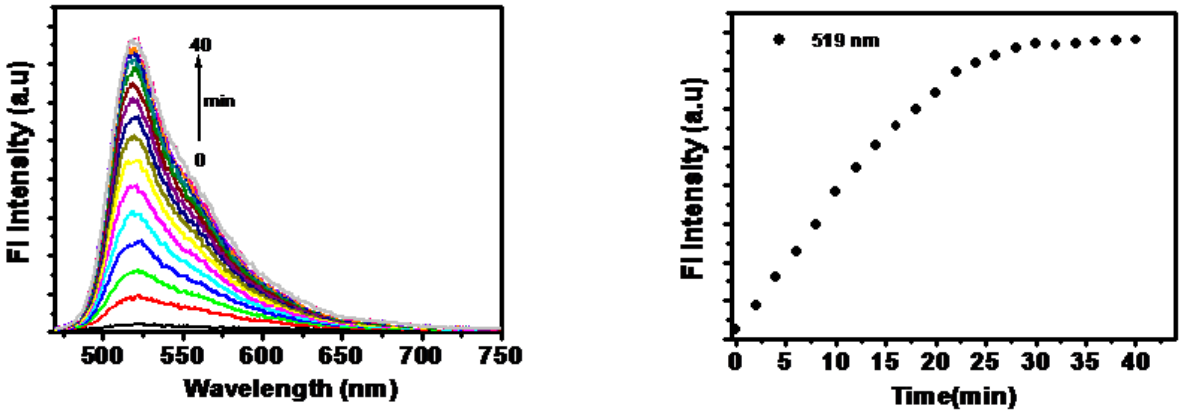


Figure 17. (a) Time dependent fluorescence change obtained for a mixture of Probe **D** (10 μM) and Au^{1+} (100 μM) in CH_3CN: PBS buffer (1:1, 0.1M, pH-7.4). $\lambda_{exc} = 452$ nm. (b) Plot of fluorescence intensity at 519 nm against time.

We recognized that probe **5a** which contains ester group could potentially be hydrolyzed by cellular esterase. We therefore tested the stability of probe **5a** in the presence of enzyme esterase and the results suggested that the probe **5a** was quite stable toward esterase.

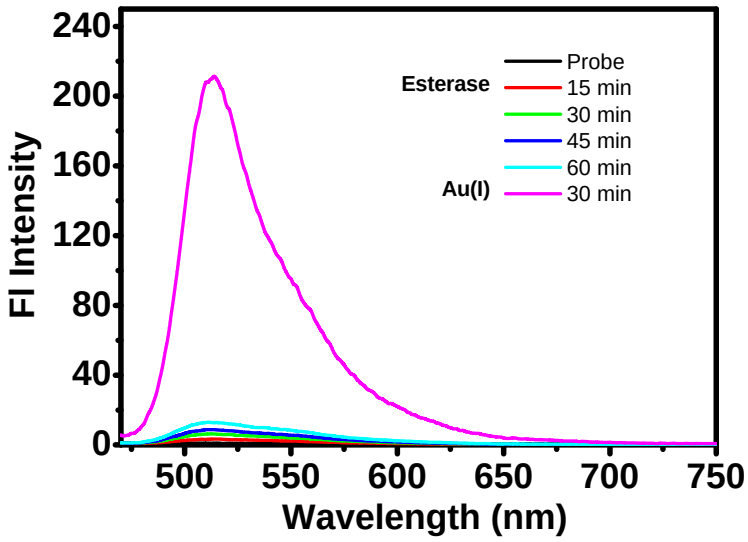


Figure 18. 6 μL of probe **5a** (5 μM) is mixed with 25 μL of esterase enzyme (0.013g/mL) stock solution (**Porcine liver esterase source -Sigma Aldrich E3019-3.5 KU**). The resultant solution is made up to 2 mL with phosphate buffer (pH = 7.4, 0.1M). The fluorescent intensity was measured at different time intervals up to 1hr. Then 100 μM of Au^{1+} is added to the mixture and intensity was measured after 30 min. ($\lambda_{excit} = 452$ nm).

4. Biology Section

Materials: The human lung cancer cell line (A549) was purchased from American Type Culture Collection (Manassas, VA). Dulbecco's modified eagle medium (DMEM), dulbecco's phosphate buffered saline (DPBS), fetal bovine serum (FBS), penicillin/streptomycin and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich, USA.

Sample preparation: The solutions of probe and AuCl were made in sterile DMSO solvent with stock solution concentrations of 30 mM and 10 mM respectively. The freshly prepared stock solutions were used for cell culture experiment.

Cell culture: A549 cells were cultured in DMEM complete media containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C incubator with 5% CO_2. After 70% confluence, the cells were seeded into 24-well plate for fluorescence microscope study.

Fluorescence Microscopy: In order to detect the Au (I) in live lung cancer cells, 2 x 10^4 A549 cells/mL were cultured in 24-well plate for 24 hours at 37 °C humidified incubator with 5% CO_2 in DMEM media. Next day, the cells were incubated with 20 μM of Au^{1+} for 1 hour and washed two times with DPBS to remove the physically unbound Au^{1+} material from the surface of cell membrane. Then the cells were incubated with 50 μM probe (**5a**) for 2 hours and again washed with DPBS for two times to remove the remaining probe. Finally, the cells were stained with 1 μg/mL of DAPI solution (for staining of nucleus) for 30 minutes and washed three times with DPBS. The fluorescence images of A549 cancer cells treated with Au(I) and probe were observed by an fluorescence microscope (Olympus IX71, Olympus U-CMAD3, T2 Tokyo Japan) through excitation of 488 nm (blue) and emission of 525 nm (green). DAPI stained A549 cells were observed through the blue channel.